BACTERIOLOGICAL REVIEWS, Sept. 1977, p. 595-635
Vol. 41, No. 3

Printed in U.S.A.

Genetics of Antibiotic Production

D. A. HOPWOOD* AND M. J. MERRICK†

John Innes Institute, Colney Lane, Norwich NR4 7UH, England

† Present address: ARC Unit of Nitrogen Fixation, The Chemical Laboratory, The University of Sussex, Brighton, BN1 9QJ, England.

INTRODUCTION

The explosion of microbial genetics over the last 30 years coincided with an equally spectacular increase in the production of antibiotics. Yet knowledge of the genetics of antibiotic production, and even of the biosynthetic pathways of antibiotics, is still disproportionately small. In part this arises from the fact that, aside from mutagenesis, which has been employed on a huge scale, genetic approaches to the improvement of strains for industrial antibiotic production have been largely ignored (236). Important fundamental genetic knowledge has, therefore, not been gained from applied microbiology as it has from plant breeding (204). Conversely, academic geneticists have not chosen antibiotic biosyntheses as models for studies of gene-enzyme relationships or of the regulation of biochemical pathways. Perhaps there was a feeling that no principles would emerge that could not be better revealed by the intensive study of synthesis or catabolism pathways of molecules such as amino acids or sugars in species of *Escherichia, Salmonella, Neurospora, Aspergillus,* or *Saccharomyces* with their highly developed experimental genetics. This concentration of effort was highly successful. However, a more diversified effort is now appropriate. For example, enteric bacteria inhabiting the human gut are clearly not completely representative of bacteria as a whole in their metabolic regulation, and much can be learned from other kinds of bacteria, such as actinomycetes or bacilli. Moreover, regulatory systems that ensure a constant supply of amino acids for protein synthesis during steady growth may differ from those controlling the synthesis of antibiotics, which typically appear at defined stages in the life cycle.

There are problems in studying the genetics of antibiotic production, but they are not insurmountable. Very few antibiotic-producing microorganisms have well-developed genetic systems, but most belong to genera with a genetically well-known member; thus, genetic analysis could probably be extended to them. Moreover, three "academic" organisms with well-developed genetic systems and representing the three major groups of antibiotic producers, *Aspergillus nidulans, Streptomyces coelicolor,* and *Bacillus subtilis*, are known to produce antibiotics; these antibiotics are, therefore, open to genetic investigation. The enzymes of antibiotic synthesis may occur in low concentrations, be part of multienzyme complexes, or be difficult to isolate for other reasons. However, this is often true of biosynthetic enzymes for primary metabolites; such enzymes have often been purified or studied in other ways once their role was identified by the isolation of a suitable mutant. Finally, the synthesis of most antibiotics is not easily studied in the steady-state conditions of an exponentially growing culture of a unicellular microorganism, since they are associated with differentiation, and this is undoubtedly an experimental obstacle to the study of the regulation of antibiotic synthesis.

A start has been made in the study of the control of antibiotic production in several diverse experimental systems, filamentous fungi, actinomycetes, and sporulating eubacteria. Sophisticated chemical, biochemical, and genetic approaches have been used, but the most complete biochemical studies have not coincided with the most extensive genetics, to the detriment of both disciplines. We hope that this article will lead to an increased awareness of the biologically and industrially interesting problems ripe for solution in this area by the combined use of biochemical and genetic techniques.

INCIDENCE OF ANTIBIOTIC PRODUCTION

General

Many groups of organisms produce substances toxic to microorganisms: for example, invertebrates, algae, and higher plants (27). However, to be reasonably coherent, we shall limit discussion to the three groups whose members produce antibiotics with some commercial or significant experimental application: the eucaryotic fungi and the procaryotic actinomycetes and eubacteria (27, 231). Moreover, although some definitions of the word antibiotic would include bacteriocins (38, 244,

277), we shall exclude them and regard an antibiotic as a compound produced by stepwise biosynthesis, rather than by the template processes of protein synthesis, and inhibitory to organisms outside the immediate relatives of the producing microbe.

Fungi

Although the first important antibiotic, penicillin, came from a filamentous fungus, fewer than a quarter of the more than 3,000 antibiotics since described are produced by fungi. The *Aspergillaceae*, including *Penicillium* and *Aspergillus*, produce the great majority of important fungal antibiotics, although recently the *Moniliales* have yielded most of the new compounds (27). Only 10 natural fungal antibiotics have been made commercially: penicillins G, V, and O, cephalosporin C (CPC), griseofulvin, fumagillin, fusidic acid, siccanin, variotin, and xanthocillin; of these only grisefulvin, fusidic acid, and the β-lactam antibiotics are significant clinically.

The development of the semisynthetic β-lactam antibiotics, with improved therapeutic properties, has maintained the penicillins and cephalosporins as one of the most widely used groups of antibiotics. More than 20 semisynthetic penicillins have found a place in chemotherapy. They are usually made by chemical addition of a new side chain to 6-aminopenicillanic acid (6-APA), originally isolated from fermentations of *Penicillium chrysogenum*, but now produced by chemical or enzymatic hydrolysis of benzylpenicillin (penicillin G). 7-Aminocephalosporanic acid (7-ACA) is made chemically from CPC; some eight semisynthetic cephalosporins are in clinical use, all chemical modifications of 7-ACA. In contrast to the formation of the classical penicillins (G, O, and V), the addition of side chain precursors to the medium does not influence the antibiotics formed by *Cephalosporium acremonium*: they are invariably CPC and penicillin N (PCN).

It is interesting that the four main fungal antibiotics are all produced by more than one genus. Penicillin, first described in *Penicillium notatum*, is made commercially by strains of *Penicillium chrysogenum*. Penicillins have been isolated from several species of *Aspergillus* and *Cephalosporium* and at least six other genera (159), and PCN has also been isolated from streptomycetes (219). CPC, produced industrially by *C. acremonium*, is also made by species of *Emericellopsis* and several other genera (159). Several new β-lactam derivatives come from *Streptomyces* species (219, 275) and deacetoxycephalosporin C from *Streptomyces* species and a variety of fungi (122). Fusidic acid was first isolated from *Fusidium coccineum* (109), a fungus closely related to the cephalosporia, and later from *Mucor remannianus* (290) and *Isaria kogana* (123). Griseofulvin was originally found in *Penicillium griseofulvum* and subsequently in about 12 other *Penicillium* species including *P. janczewski* and *P. patulum*; mutant strains of *P. patulum* are used to produce it commercially. A completely unrelated organism, *Khuskia oryzae*, also makes griseofulvin (108).

Of the remaining commercial fungal antibiotics, fumagillin is made by *Aspergillus fumigatus* (31), siccanin by *Helminthosporium siccans* (124), variotin by *Paecilomyces varioti* (279), and xanthocillin by *P. notatum* (1).

Actinomycetes

This group is unique in the number of antibiotics made from it, and in the diversity of these antibiotics' chemical structures and modes of action. Perlman (231) lists nearly 70 that are produced commercially. There is a compilation of actinomycete antibiotics (287), which is being supplemented by monthly information in the same format in the *Journal of Antibiotics (Japan)*. Bérdy (27) counts more than 1,950 streptomycete compounds, including the important chloramphenicol, erythromycin, kanamycin, neomycin, novobiocin, streptomycin, and tetracyclines, out of 2,080 actinomycete antibiotics, but this may reflect the ease of isolation of streptomycetes. More recently, *Nocardia* species (rifamycin, ristocetin) and *Micromonospora* species (gentamicin, sisomicin) have emerged as producers of important antibiotics, and attention is turning to genera such as *Actinoplanes* (23, 60, 61), *Streptosporangium* (161, 278), *Streptoverticillium* (311), and *Thermoactinomyces* (233). With increasing knowledge of actinomycete biology (64) and the realization that many organisms remain to be isolated by suitable selective procedures, the list of actinomycete antibiotics will doubtless continue to grow.

Semisynthetic actinomycete antibiotics are proportionally less important than in the fungal case but are nevertheless significant in absolute terms: for example, derivatives of kanamycin (e.g., amikacin), rifamycin (rifampin), lincomycin (clindamycin), and various tetracyclines.

Recent taxonomy suggests that the genus *Nocardia* is heterogeneous, and genetic studies (see below) support this view. *N. mediterranei*, the rifamycin producer, resembles *Streptomyces* species in its genetics, whereas *N. ery-*

thropolis and some related strains share genetic features with *Mycobacterium smegmatis*. This agrees with the idea that *N. mediterranei* is close to *Streptomyces* (it was originally named *S. mediterranei*), whereas *N. erythropolis* is closer to *Mycobacterium*, forming part of the so-called *Mycobacterium rodochrous* complex (37). It will, therefore, be interesting to investigate other antibiotic-producing *Nocardia* strains by recent taxonomic criteria, including the presence of characteristic lipids called mycolic acids found in mycobacteria and the true nocardiae but not in the so-called nocardiae represented by *N. mediterranei* (34, 215). *N. lurida*, the ristocetin producer, lacks such lipids, as does the producer of nocardin, *N. autotrophica* (110). However, caution is needed: the nocardin producer was originally named *N. coeliaca*, authentic strains of which possess the characteristic lipids. Taxonomic and genetic studies must be done on strains that actually produce antibiotics. Such studies have not been made on strains producing the other nocardia antibiotics listed by Umezawa (287), although strains of some of the relevant species (*N. formica, N. gardneri*) appear to lack mycolic acids, and none of these species is known to possess such compounds. Thus there is the interesting possibility that most, if not all, of the nocardiae that produce antibiotics belong to the streptomyces-like organisms. This would have important implications for genetic investigations of antibiotic production.

Eubacteria

Bérdy (27) credits the eubacteria with about 360 antibiotics scattered through many taxonomic groups. However, bacilli produce nearly half the total and pseudomonads nearly a quarter. The genus *Bacillus* is by far the most significant commercially, nearly all the interesting eubacterial antibiotics being cyclic or linear peptides produced by members of this genus (250): *B. licheniformis* (bacitracin), *B. brevis* (gramicidin S and linear gramicidin A tyrocidine, edeines), *B. polymyxa* (polymyxin), *B. colistinus* (colistin), *B. subtilis* (mycobacillin). Another *Bacillus* product of interest is butirosin (*B. circulans*), the only aminocyclitol known to be produced by a non-actinomycete (55). Of the many *Pseudomonas* antibiotics, only pyocyanine (*P. aeruginosa* [183]) and pyrrolnitrin (*P. aureofaciens*) have some commercial interest. The cyclic polypeptide nisin is produced by *Streptococcus lactis*. Other eubacterial antibiotics appear to have neither commercial nor genetic relevance at the present time. However, perhaps it is worth noting two recently described myxobacterial antibiotics (232, 289) because of current interest in the developmental genetics of myxobacteria.

GENETIC SYSTEMS AVAILABLE IN ANTIBIOTIC-PRODUCING MICROORGANISMS

General

Even without genetic analysis, biochemical studies of mutants help to explain the biosynthesis of antibiotics. However, genetics is then contributing a small fraction of its analytical potential. For example, evidence that a mutant differs from its progenitor by a single mutation normally depends on observing no other phenotypes besides those of the mutant and wild type in a suitable cross; genetic analysis, embodying some kind of recombination test, is needed to determine the arrangement of the genes involved in antibiotic synthesis (whether they are scattered or clustered, chromosomally or plasmid borne, etc.); a genetic complementation test is the best criterion for classifying mutants before embarking on biochemical studies of representative examples; dominance or gene dosage tests help to determine the roles of regulatory genes; and studies of genetic fine structure could illuminate structure-function relationships of individual proteins or multienzyme complexes involved in antibiotic biosynthesis. A perfect genetic system would embody all these tests and possibly others. Very few organisms have been developed as ideal genetic subjects, and, as far as antibiotic producers are concerned, the fungus *Aspergillus nidulans*, the actinomycete *Streptomyces coelicolor*, and the eubacterium *Bacillus subtilis*, all "academic" organisms known to produce antibiotics, come nearest to the ideal though still falling short of it. However, systems of gene exchange are widespread in the microbial groups that produce antibiotics, so the potential for genetic analysis is enormous.

Eucaryotes (here represented by the fungi) and procaryotes (actinomycetes and eubacteria) differ significantly in their genetics. The deoxyribonucleic acid (DNA) of eucaryotes occurs in several separate chromosomes together with structural and regulatory (histone) proteins; complex nuclear division mechanisms (mitosis and meiosis) ensure the exact partitioning of genes to daughter nuclei and progeny; and the chromosomes are retained in a discrete region of the cell by a nuclear membrane. The chromosome of procaryotes, on the other hand, is a single circular DNA molecule with few, if any, protein molecules permanently associated with it, and there is no nuclear membrane. Procar-

yotes possess plasmids (86, 213)—circular DNA molecules considerably smaller than the chromosome and representing genes dispensable, at least under certain conditions, to the organism carrying them. Such genes determine characters—sex, antibiotic production or resistance, catabolic proficiency, pathogenicity, etc.—that are important for the evolutionary versatility of the population but are carried by only a proportion of its members. Plasmids will have many applications in the experimental manipulation of antibiotic-producing organisms (138). Eucaryotes usually, though not always, have regular sexual cycles involving fusion of whole nuclei and subsequent reassortment of chromosomes from the fusion nucleus; on the other hand, procaryotes indulge in several processes, transformation, transduction, and conjugation, with the same genetic consequences as sexual reproduction—the creation of new combinations of genes—but differing markedly from it. Probably because of differences in chromosome structure, eucaryote chromosomes readily undergo rearrangement of segments to give inversions and interchanges, whereas those of procaryotes do not. The resulting conservation of gene arrangements in procaryotes has the useful consequence that crosses between strains representing divergent lines of selection from a common ancestor are not hindered by inhomology between chromosomes. Finally, although many details of the storage, transmission, and expression of genetic information (DNA replication, transcription, and translation) are the same in procaryotes and eucaryotes, there are also differences, particularly in the signals initiating and terminating these processes (175, 176, 297). These differences present barriers, currently under attack in many laboratories, to the useful transfer of genes between eucaryotes and procaryotes.

Fungi

Recombination has been described in six species of antibiotic-producing fungi, all members of the *Plectomycetales* (Table 1). Only three of these species, *A. nidulans*, *Emericellopsis salmosynnemata*, and *Emericellopsis terricola*, have a conventional sexual cycle. All are homothallic, so that many of the perithecia (correctly termed cleistothecia) arise by self-fertilization. The asci in individual perithecia are generally all selfed or all crossed; hence, a single perithecium can usually be diagnosed as of crossed origin by analyzing a sample of its ascospores, most conveniently by the use of conidial color mutations in one or both parents (238). Discrete asci containing eight ascospores are present only in immature perithecia; at maturity, the ascus wall breaks down so that orthodox tetrad analysis is not normally carried out in these fungi, although it can be done. Analysis of random spores from a single hybrid perithecium allows the detection of linkage between markers and of polygenic segregation and epistasis. Fine mapping can be done by selective plating of random spores.

Several types of genetic analysis—tests of dominance and complementation, centromere mapping, and detection of linkage groups—are routinely carried out by "parasexual" analysis even in organisms with a regular sexual cycle. It is the only system available in the imperfect fungi *P. chrysogenum*, *P. patulum*, and *C. acremonium*. The life cycle of all these fungi is predominantly haploid, with occasional heterokaryon formation at frequencies varying significantly between species. From these heterokaryons occasional diploid nuclei may be selected as first demonstrated by Roper (248) in *A. nidulans*.

Roper's technique, applicable to any mold with uninucleate conidia, has been successfully applied to several fungi. A prototrophic heterokaryon is formed on minimal medium from two nutritionally different auxotrophs. All haploid conidia formed by the heterokaryon are of one or the other auxotrophic type and fail to grow on minimal medium. However, a diploid conidium resulting from fusion of unlike nuclei is

TABLE 1. *Antibiotic-producing fungi in which genetic recombination has been demonstrated*

Species	Antibiotic	Type of recombination	References
Aspergillus nidulans	Penicillin G	Sexual, parasexual	238, 127, 128
Cephalosporium acremonium	CPC PCN	Parasexual	223
Emericellopsis salmosynnemata	PCN CPC	Sexual, parasexual	88
Emericellopsis terricola var. *glabra*	PCN CPC	Sexual	87
Penicillium chrysogenum	penicillin G,O,V	Parasexual	239
Penicillium patulum	griseofulvin patulin	Parasexual	49

prototrophic; in *A. nidulans*, plating of conidia from a heterokaryon yields diploids at a frequency of 10^{-6} to 10^{-7}.

Diploid strains of *A. nidulans* can be distinguished from haploids by their larger conidial diameter (238), but this is not always a reliable indicator of ploidy in *P. chrysogenum* (191, 239) or *P. patulum* (49). The introduction of major mutations into strains can have considerable effects on conidial size (84), and, therefore, conidial diameter and DNA content should be correlated for accurate ploidy determination, and diploid strains should always be compared with their immediate parents.

Genetic analysis by the parasexual cycle depends on the isolation from heterozygous diploids of two kinds of vegetative segregants. (i) Diploids homozygous at one or more loci that were originally heterozygous arise by mitotic crossing-over between chromatids of homologous chromosomes, followed by appropriate segregation of chromatids at the next nuclear division. Homozygosity occurs for all loci distal to the point of crossing-over on that chromosome arm, and so analysis of such diploids allows ordering of markers on the same chromosome arm, estimation of relative mitotic crossover frequencies between them, and mapping of the centromere if markers are available on both chromosome arms. (ii) Haploids (and aneuploids that usually tend to be selected against) arise by nondisjunction at diploid mitosis with random assortment of chromosomes seldom accompanied by recombination within linkage groups. Hence, lack of segregation of two loci after haploidization indicates that these loci are probably on the same chromosome.

Either heterokaryons or heterozygous diploids may be used for dominance and complementation tests, although the irregular nuclear constitution of heterokaryons may preclude rigorous tests, and, therefore, heterozygous diploids are probably preferable. For further details of genetic analysis by the sexual and parasexual cycles, see Fincham and Day (91) and Hopwood (135).

The parasexual cycle occurs in *P. chrysogenum*, *C. acremonium*, and *P. patulum*, and problems of genetic analysis have been found in all three species. However, in many cases where the parasexual cycle has been examined in an imperfect fungus, the parent strains have been "improved" mutants selected after mutagenic treatments. Hence, some difficulties may well be due to mutations or chromosome aberrations induced during these treatments. Certain problems, however, may be due to heritable characteristics such as the ability to form heterokaryons, which, for example, varies markedly among aspergilli. Hence, whereas heterokaryons in *A. nidulans* are invariably "forced" by the use of auxotrophic markers, in *Aspergillus amstelodami* heterokaryons (assessed by formation of mixed conidial heads) readily arise between prototrophic strains (C. E. Caten, personal communication). To establish heterokaryons in the penicillia and cephalosporia, one must usually mix conidia of the two auxotrophic parents on supplemented minimal medium (223) or complete medium (191) and allow growth for 7 to 10 days, after which the mycelial mat is broken up and plated on or in minimal agar medium.

Such problems of poor heterokaryon formation may well be overcome by the use of induced protoplast fusion, which has recently been demonstrated in *A. nidulans*, *Aspergillus niger*, *P. chrysogenum*, *P. notatum*, *P. patulum*, and *C. acremonium* (13, 90) and also between *Penicillium roquefortii* and *P. chrysogenum* (12).

Diploids have proved particularly difficult to isolate in *C. acremonium* (223), *P. patulum* (49), and *E. salmosynnemata* (87). In *C. acremonium* and *P. patulum*, frequent small-spored prototrophic colonies arose as sectors from heterokaryons or by plating spores from heterokaryons. The prototrophs could not be induced to segregate by treatment with *p*-fluorophenylalanine (PFA), an effective haploidization agent in fungi (177). It seems likely that unstable diploid clones had arisen within the heterokaryotic colony and that the prototrophic colonies were the products of mitotic segregation and haploidization in these diploids. Variations in the "classical" parasexual cycle as found in *A. nidulans* or *P. chrysogenum* may well be found as more imperfect fungi are studied. An extreme example occurs in *Humicola* species (66) where heterozygous diploids were isolated directly from mixed cultures of auxotrophs without any detectable intervening heterokaryotic state.

When diploids were eventually isolated in *C. acremonium* (223) and *P. patulum* (49), they were extremely stable and did not segregate spontaneously, although haploidization was achieved by treatment with PFA. The haploid segregants in *C. acremonium* (223) showed apparent random assortment of markers, although the recombinant double auxotrophs occurred at a very low frequency, which was attributed to poor germination. In *P. patulum* (49), haploid segregants occurred very rarely, and, therefore, no genetic analysis was carried out. Recombinant genotypes were obtained from a presumptive heterozygous diploid in *E.*

salmosynnemata (87), but the nonrecovery of some recombinant types and the lack of conidial measurements makes it impossible to be sure if haploidization had occurred.

The parasexual cycle has been applied more extensively in *P. chrysogenum* (20, 21, 83, 189–193) where diploids can be obtained at frequencies of 10^{-6} to 10^{-7} and show a low frequency of spontaneous segregation.

In early studies with "improved titer" strains of *P. chrysogenum* (83, 192, 193, 263), most haploid segregants from diploids were of one or the other parental genotype, a phenomenon termed "parental genome segregation" (192). This was probably due to the parent haploids differing in chromosomal rearrangements such as reciprocal translocations, which prevented random chromosome assortment. By using closely related "sister" strains as parents, these barriers to recombination were overcome, and random assortment of "haploidization groups" (presumably chromosomes) was achieved (20).

Since spontaneous mitotic recombination and haploidization are both rare in *P. chrysogenum* (20), it is convenient to induce segregation with chemical and physical agents. Nitrous acid, nitrogen mustard, ultraviolet light (UV), and X rays all significantly increased segregation from a heterozygous diploid, although effects on mitotic crossing-over and on haploidization could not be clearly distinguished (186). UV, ethylenimine, nitrous acid, and 5-fluorouracil were tested for their ability to increase mitotic crossing-over specifically, and ethylenimine and 5-fluorouracil both increased it more than 15-fold (K. B. Morrison and C. Ball, Abstr. 2nd Int. Symp. Genet. Ind. Microorg. 1974, p. 83). PFA increased haploidization in *P. chrysogenum* and also significantly reduced selection against certain alleles (20). Other chemical agents such as benlate (121) are also good haploidizing agents; benlate was more effective than PFA for haploidization in *P. chrysogenum* (G. F. St. L. Edwards, I. D. Normansell, and G. Holt, Aspergillus Newsletter 12, 1975, p. 15). The antibiotic griseofulvin induced haploids in *Coprinus lagopus* (222) and may also be effective in other fungi. When haploidization has been achieved, other chemicals such as *N*-glyceryl polyfungin can be used to select the haploids (18).

Actinomycetes

Most actinomycetes produce antibiotics. We shall, therefore, review the genetics of the group, since interesting compounds may well be discovered in the relatives of antibiotic nonproducers that have been studied genetically. Recombination is very widespread, having been reported in the genera *Streptomyces, Nocardia, Micromonospora, Mycobacterium*, and *Thermoactinomyces*. We are not aware of genetic studies in *Actinoplanes, Streptosporangium*, or *Streptoverticillium*, but increased interest in antibiotics produced by these organisms may lead to genetic studies.

In mesophilic organisms of the genera *Streptomyces, Nocardia*, and *Micromonospora*, there is no firm evidence for transduction or transformation; some preliminary reports of transformation in *Streptomyces* (13) and one of transduction (10) have not been confirmed or extended. With the exceptions of transformation in *Thermoactinomyces vulgaris* (see below) and of some limited studies of transduction in *Mycobacterium* species, known genetic interactions in actinomycetes are currently confined to naturally occurring conjugation phenomena and to artificially induced protoplast fusion (146a). However, very few serious attempts have been made to develop systems of genetic analysis for streptomycetes based on transduction or transformation, and the existence of such systems is, therefore, not precluded. Transfection of protoplasts by bacteriophage DNA and conditions for regenerating protoplasts into viable mycelia have been achieved (224, 226, 227), so that useful systems for introducing biologically active DNA into these organisms may be potentially available.

Transformation in *Thermoactinomyces*. Typical transformation occurred in the thermophilic *Thermoactinomyces vulgaris* (144), with transformation frequencies per marker up to 10^{-3} when donor DNA was added to growing recipient cultures, or when donor and recipient were grown together. Recipients became competent at a particular stage in mycelial growth, possibly associated with the onset of sporulation. More than half of a sample of 20 wild types were competent (139, 144); of the rest, a small minority were unable to act as DNA donors (or recipients) in mixed-growing cultures, and they excreted deoxyribonuclease in amounts probably sufficient to abolish transformation (139). It is suggestive that typical transformation occurs in *T. vulgaris* with an endospore structure (65) and a DNA base composition (273) similar to bacilli; it supports the view that *T. vulgaris* and its relatives are distinct evolutionarily from most other groups of actinomycetes.

Conjugation in other genera. In mesophilic actinomycetes, the morphological basis of gene exchange is unknown, but conjugation is indicated by two observations: contact between viable (and, in practice, growing) cells or mycelia

is required for gene exchange, and the genetic consequences are those characteristic of conjugation, since groups of linked markers from each parent are typically inherited together. The involvement of plasmid sex factors in *S. coelicolor* A3(2) (see below) may imply a closer similarity to conjugation in gram-negative eubacteria than might be expected from the taxonomic gulf between the two groups. Heterokaryons, which are common in some streptomycetes, perhaps arise by a different mechanism (see below).

There is at least one significant difference between conjugation in *Streptomyces* species, *Micromonospora* species, and *Nocardia mediterranei* on the one hand and other *Nocardia* species (*N. erythropolis*, *N. canicruria*, and *N. restrictus*) and *Mycobacterium smegmatis* on the other: each examined strain of the latter group was self-sterile but fertile in crosses with other strains (*N. erythropolis* × *N. canicruria* [2], *N. canicruria* × *N. restrictus* [C. Vezina, Abstr. 1st, Int. Symp. Genet. Ind. Microorg. 1970, p. 167], and interstrain crosses of *M. smegmatis* [284]). Genetic analyses of interstrain crosses may have complicated interpretation of the *Nocardia* data (2). A divergence of genetic behavior within the genus *Nocardia* reflects the idea (see above) that *N. erythropolis*, *N. canicruria*, and *N. restrictus* represent part of a "cluster" of strains within "*Mycobacterium rodochrous*" (64), whereas *N. mediterranei* is taxonomically close to *Streptomyces*. Since the self-sterile strains are not known to produce important antibiotics, we shall not consider their genetics further.

Conjugation and its consequences in *Streptomyces*. Table 2 lists genetic phenomena in *Streptomyces* other than *S. coelicolor* A3(2). There are many reports of recombination, but in some cases, unfortunately including producers of four major antibiotics—chlortetracycline (*S. aureofaciens*), erythromycin (*S. erythreus*), neomycin (*S. fradiae*), and streptomycin (*S. griseus*)—the data did little more than establish its existence. Another early report, in *S. griseoflavus*, included the intriguing possibility of a regular diploid or dikaryotic state of the genes in spores in contrast to the haploidy of most species, but these studies, also, have not been extended. For one of the other species, *S. olivaceus*, a series of results resembling those of the earlier studies in *S. coelicolor* A3(2) has appeared, mainly in Ukrainian, but with a recent English summary (205). This included the remarkable claim that the hairy projections on the spore surface, interpreted by others as inert appendages (306), are sexual organs analogous to the pili of enteric bacteria. A priori, mating between spores seems unlikely; moreover it was excluded in *S. coelicolor* A3(2), whose spores in any case are smooth (306); gene transfer occurred only after incubation of spores long enough for germination (136).

S. coelicolor A3(2) is the strain in which the great majority of genetics has been done (140, 264) and the only one in which plasmid fertility factors have been recognized (29, 140, 145, 240, 293). It probably provides a good model for other strains, even though some differences, at least with respect to heterokaryon and heteroclone formation, are already apparent between strains (see below). The marked similarities of gene sequence on the circular linkage maps of the species so far examined may in any case indicate that they are rather closely related. In view of the production of two antibiotics by *S. coelicolor* A3(2), one (actinorhodin) chromosomally determined (308) and the other (methylenomycin) by plasmid-borne genes (164, 307), this organism offers good possibilities for the study of the genetic control of antibiotic synthesis.

Linkage analysis in *Streptomyces*. The commonest system of linkage analysis in *Streptomyces* is the selection of haploid recombinants from mixed cultures of genetically marked parents allowed to sporulate by growth on nonselective solid medium for several days. Nonselected marker segregation in samples of the selected progeny allows very efficient gross mapping since the linkage maps of the species so far studied are short; hence, all chromosomal markers show linkage.

A general mapping procedure for preliminary study of a new strain was described by Hopwood (130, 134, 135). It was applied successfully in *S. bikiniensis* (57), *S. rimosus* (96), *S. glaucescens* (25), *Streptomyces* sp. 3022a (possibly *S. venezuelae*) (94), and *S. acrimycini* (D. A. Hopwood and H. M. Wright, unpublished data). The products of crosses between parents who each carry two selectable alleles were plated on four differently supplemented media; the data immediately indicated circular linkage of the four markers, providing the basis for further analysis by particular selections as outlined below.

An interesting alternative approach, adopted in *S. venezuelae* to overcome the problem of reversion of selective alleles in poorly fertile crosses (3), was to disregard all recombinants differing from a parent by a single marker (since they might have included revertants),

TABLE 2. *Genetic phenomena in streptomycetes other than S. coelicolor A3(2)*

Species	Antibiotic	Genetic phenomena	References
S. achromogenes var. *rubradiris*	Rubradirin	Recombination; heteroclone analysis yielded circular map	56
S. acrimycini	Unidentified	Heterokaryosis and recombination; haploid selection yielded circular map	Hopwood and Wright[a]
S. albus		Heterokaryosis	40
S. antibioticus	Actinomycin	Heterokaryosis and recombination	294
S. aureofaciens	Chlortetracycline; tetracycline	Recombination	147
		Interspecific recombination with *S. rimosus* and *S. coelicolor*	4, 6, 235
S. bikiniensis var. *zorbonensis*	Zorbamycin; zorbonomycin; etc.	Recombination; haploid selection yielded circular map	57
S. clavuligerus	Cephamycins	Recombination	Aharonowitz and Demain[b]
S. coelicolor (other strains)		Heterokaryosis and recombination	141
S. cyaneus		Heterokaryosis	39
S. erythreus	Erythromycin	Recombination	67, 162
S. fradiae	Neomycin	Recombination	40
S. glaucescens		Heterokaryosis and recombination; haploid selection yielded circular map; melanin plasmid	25 26
S. griseoflavus		Recombination	252
S. griseus	Streptomycin; ? others	Heterokaryosis	39
		Recombination	40
		Interspecific recombination with *S. coelicolor*	183
S. hydroscopicus	Turimycin	Turimycin plasmid	157
S. kasugaensis	Aureothricin; kasugamycin	Antibiotic plasmid(s)	225
S. lipmannii	Cephamycins	Heterokaryosis	Aharonowitz and Demain[b]
S. lividans		Recombination	143, 144
		Receives SCP1 and SCP1′ from *S. coelicolor*	143
		Interspecific recombination with *S. coelicolor*	11
S. olivaceus		Recombination; haploid selection and heteroclone analysis yielded circular map	205
S. parvulus	Actinomycin	Receives SCP1 and SCP1′ from *S. coelicolor*	Hopwood and Wright[a]
S. rimosus	Oxytetracycline	Heterokaryosis and recombination; haploid selection or heteroclone analysis yielded circular map;	5, 96
		Oxytetracycline plasmid;	36
		Interspecific recombination with *S. coelicolor* and *S. aureofaciens*	4, 6, 235
S. scabies		Heterokaryosis and recombination; tyrosinase plasmid	113, 114
S. sphaeroides		Heterokaryosis	40
S. venezuelae (and *Streptomyces* sp. 3022a)	Chloramphenicol	Heterokaryosis	40
		Recombination; haploid selection yielded circular map.	3, 94
		Chloramphenicol plasmid	3

[a] D. A. Hopwood and H. M. Wright, unpublished data.
[b] Y. Aharonowitz and A. L. Demain, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, O22, p. 183.

and to choose the circular sequence of markers that minimized quadruple (or sextuple) crossovers. The approach was valid because the excluded classes could arise by the simplest crossover pattern, that is, a double crossover on a circular arrangement, irrespective of marker sequence, and, therefore, did not contribute sequence information.

Once the positions of some markers are known, an efficient approach is selection for alleles at two loci on opposite sides of the linkage map with one or more nonselected markers of known map location in each of the two arcs between. The data are analyzed for linkage by a two-stage process, considering first the allele ratios and second the segregation of the unknown locus with respect to possible neighboring loci (133, 135). This approach was used in *S. rimosus* (96), *S. glaucescens* (25), and *S. olivaceus* (205) in addition to *S. coelicolor*. For finer mapping, the most efficient procedure is usually selection for recombination between outside markers (139).

In *S. coelicolor* A3(2), recombination in crosses between certain mixtures of fertility types with respect to the SCP1 plasmid is so frequent (up to 100% of progeny) that no selection is necessary; samples of total progeny can be analyzed for linkage.

In *S. coelicolor* A3(2) (267, 268) and *S. olivaceus* (205) merozygotes became progressively more complete as incubation time of the cross increased, over periods of many hours, and attempts were made to interrupt the process experimentally. However, there appears to be no example so far of linkage analysis by this procedure.

Heteroclones in *Streptomyces*. Heteroclones are colonies developing on selective medium from partially diploid units and containing mixtures of recombinant and parental genotypes. In their simplest form, their founding genome could arise by single crossing-over within a merozygote between a circular chromosome of one parent and a linear fragment from the other to generate a terminally redundant genome (133). Crossing-over at various positions within the duplicated segment during subsequent growth of the colony generates a family of recombinant spores. Colonies derived by nonselective plating of these provide data for the calculation of map intervals, information not easily obtained from haploid analysis. This method established the spacing of markers on the linkage maps of *S. coelicolor* A3(2) (131–133, 141), *S. rimosus* (5, 6), and *S. olivaceus* (205).

Whether or not the model outlined above explains the origin of all heteroclones has been questioned (264). Certainly not all segregation data fit the model, especially in heteroclones selected to be heterozygous over long regions (264), but distortions in the frequencies of recombinant classes due to segregation and competition within the growing colony explain many of the apparent discrepancies (134).

The original method for heteroclone selection (266) required two closely linked markers, one in each parent. A cellophane transfer method that renders this unnecessary was later devised for *S. coelicolor* A3(2) (265), and adopted for *S. rimosus* (5, 6).

In *S. achromogenes* var. *rubradiris*, little progress could be made in mapping by haploid selection, nor could typical heteroclones of the *S. coelicolor* type be recovered (56). Evidence of gene sequence was based primarily on the patterns of heterozygosity of groups of markers in the atypical heteroclones that arose.

Heteroclones, by developing on selective media, provide dominance and complementation tests for nutritional and resistance or sensitivity mutations (119, 120, 141), but whether or not they can be harnessed to the study of antibiotic genes remains to be seen; probably plasmid primes will be more useful (see below).

Heterokaryons in *Streptomyces*. Colonies conforming to the operational definition of fungal heterokaryons occur in streptomycetes (39); they grow on selective medium but constantly segregate both parental genotypes. In some strains, no true recombinants were found (39, 40), but in others, heterokaryons occurred in varying proportions alongside recombinants: for example, in *S. rimosus* (96) and *S. glaucescens* (25). In *S. rimosus*, heterokaryons were found only when the selective markers were rather leaky (96); growth on the selective plates was presumably needed for their formation. In *S. coelicolor* A3(2), heterokaryons occur very rarely among samples of selected recombinants (141) but were recovered in large numbers by transferring mixed growth of complementary auxotrophs growing on cellophane from nonselective to selective medium (249, 264).

In some cases, heterokaryons hindered genetic analysis by obscuring the rarer recombinants that provided mapping information; indeed, it is conceivable that rare recombinants might have been overlooked in those strains in which exclusively heterokaryons were detected. However, heterokaryons can also be useful analytically. Their formation on selective medium provided a complementation test for auxotrophs in *S. coelicolor* A3(2) (249) and allowed recovery of newly induced auxotrophic

mutations in comutation studies in *S. coelicolor* A3(2) and *S. rimosus* (51). In *S. scabies*, heterokaryons between melanin-producing and melanin-nonproducing strains segregated (almost) exclusively melanin-producing clones, providing evidence for extrachromosomal determination of melanin production (114). Now that plasmid involvement in the control of various characters in *Streptomyces* is being established (see below), it will be interesting to see if heterokaryons will provide a clear test for plasmid involvement.

The nature of heterokaryons in these procaryotes is an intriguing problem that has not been seriously studied. The challenge is to imagine a mechanism allowing the maintenance of two complete parental genomes in a common cytoplasm without frequent opportunities for recombination. Whether or not heterokaryons and the merozygotes that lead to recombination arise by different conjugation routes is also not clear. Intuitively this seems more likely, and the balance of evidence probably points in this direction, but there is no conclusive proof (136, 264).

Plasmids in *Streptomyces*. *S. coelicolor* A3(2) is the only strain in which plasmids have been definitely implicated in gene exchange (29, 140, 145), but, in view of the similarities between streptomycetes, it seems likely that they will be found in other species. The SCP1 plasmid, which also determines methylenomycin synthesis, is responsible for a limited amount of chromosome transfer when in the autonomous state, $SCP1^+ \times SCP1^-$ crosses being approximately 10 times more fertile than $SCP1^- \times SCP1^-$ crosses. Recently a second sex factor, SCP2, has been revealed (29), responsible for nearly all of the recombination, that occurs at a frequency of 10^{-5} to 10^{-6} in $SCP1^- \times SCP1^-$ crosses. SCP1 can integrate into the chromosome in various ways (145) and is then a very efficient agent for the transfer of chromosomal segments to $SCP1^-$ strains with nearly 100% efficiency. It can also acquire chromosomal insertions to give the SCP1′ state (144–146). In principle, SCP1′ strains (or SCP2′ if these occur) provide the best hope for quantitative complementation and dominance tests, but a systematic method for their isolation has not yet been devised. DNA corresponding to SCP1 has not been isolated, precluding in vitro manipulation of the plasmid, but SCP2 has been isolated as a plasmid of molecular weight 18×10^6 to 20×10^6 (29, 257).

In other streptomycetes, genetically defined plasmids have been implicated in the production of melanin (26, 114) or antibiotics (see below), but there is no proof that they are sex factors.

Interspecific recombination in *Streptomyces*. Some empirical attempts to select interspecific recombinants have succeeded (4, 7, 182, 235). However, imperfect homology between genomes is likely to limit such recombination, and the best hope for interspecific gene transfer is probably the development of suitable plasmid vectors.

Recombination in *N. mediterranei*. Analysis of haploid recombinants in the rifamycin-producing *Nocardia mediterranei* gave results strikingly similar to those in streptomycetes, extending even to a resemblance in gene sequence on the circular linkage map (259). Although the proportion of mixed-recombinant colonies was higher than in typical streptomycete crosses, presumably because the plating units were often multinucleate mycelial fragments, this proved no serious obstacle to genetic analysis.

Recombination in *Micromonospora*. Recombination studies in *Micromonospora* (28) established a conjugation type of gene exchange in self-fertile strains of *M. chalcea*, *M. echinospora*, and *M. purpurea* but did not extend to linkage analysis.

Recombination by protoplast fusion. It has recently been shown (146a) that very efficient recombination occurs in several streptomycetes when protoplasts are artificially fused by polyethylene glycol treatment and allowed to regenerate on nonselective medium. This technique promises to be extremely useful for the development of recombination studies in new species, probably being capable of extension to members of other genera and perhaps to interspecific crosses.

Eubacteria

Bacillus and *Pseudomonas* are the significant genera of eubacterial antibiotic producers. Apparently, 66 antibiotics are produced by strains of *Bacillus subtilis* (27), two closely related strains of which have been extensively investigated genetically. A major thrust has been the analysis of sporulation (253), the genetics of antibiotic production having been studied only for its possible relationship to sporulation: most mutants blocked early in sporulation fail to produce antibiotic(s), but it is unlikely that the genes identified by such mutations are directly concerned in antibiotic synthesis. Strains producing the important *Bacillus* antibiotics, several of them studied in detail biochemically (see below), have not been developed as experimental genetic systems.

B. subtilis genetics depends on transformation and generalized transduction. Transformation and transduction, by phage SP10, are well adapted to mapping over comparatively short map intervals (about 1% of the whole genome), but for the large phage PBS1, the proportion is as high as 5 to 8%. A combination of transformational and transductional approaches has allowed a complete circular linkage map to be defined (174). A system allowing dominance and complementation testing, lacking for many years in *B. subtilis*, has recently become available for some chromosomal regions; it involves a special class of partially diploid strains (15). There are several useful reviews of techniques in *B. subtilis* genetics (309, 311).

Transformation has been studied in certain other bacilli, including strains of *Bacillus licheniformis* and *Bacillus pumilus* (309), but apparently not in relation to antibiotic synthesis.

Pseudomonas, the second most prolific genus of eubacterial antibiotic producers, is also a group in which genetic studies are fast developing. However, once again, the strains studied genetically (126, 274) have not been investigated from the standpoint of antibiotic synthesis.

Certain strains of *Pseudomonas aeruginosa* have a well developed conjugation system, whereas general transduction has been used for genetic analysis in *P. aeruginosa* and *P. putida* (126, 274). A particular interest of *Pseudomonas* genetics is the existence of plasmids, many of them self-transmissible, that control catabolic functions (99). In view of the importance of such plasmids in pseudomonads and the implication of plasmids in antibiotic synthesis in streptomycetes (see below), it would be interesting to investigate the possibility that some of the *Pseudomonas* antibiotics are plasmid determined.

Transformation has been studied in *Streptococcus* (77), but chromosomal recombination has not been related to studies of the putative nisin plasmid (see below).

The vast majority of genetic work on eubacteria, of course, concerns the *Enterobacteriaceae*, exemplified particularly by *Escherichia coli* and *Salmonella typhimurium*. This group produces some antibiotics (27) but none of importance, and no genetic studies appear to have been made on them.

SOME REMARKS ON THE PHYSIOLOGY OF ANTIBIOTIC PRODUCTION

Several concepts are relevant to antibiotic production that do not arise in studies of the genetics of intermediary metabolism. They have important bearings on the genetics of antibiotic production, particularly on its regulation and on the consequences of mutational interruption of biosynthetic pathways.

Primary and Secondary Metabolites

Although the boundary between the two areas of metabolism is imprecise, it seems useful to distinguish "primary" from "secondary" metabolism (42, 43, 286). The first concerns the synthesis of materials essential for the growth of organisms: the components of proteins, nucleic acids, carbohydrates, lipids, coenzymes, etc.; and the pathways are likely, and have indeed often been shown, to be very similar over broad groups of microorganisms; the genes involved have been conserved over long periods of evolution. On the other hand, materials such as antibiotics, which are produced only by certain groups of microbes and are often different even between apparently closely related strains, are known as secondary metabolites, at least in cases where their role in the life of the producing organism is open to argument. Not only is the genetic information for their synthesis confined to particular narrow taxonomic groups, but it is also usually, though not always, expressed only at defined stages in the life cycle of the organism—or of a batch culture in a fermentor. The growth of such a culture is often divided into an early "trophophase," when cellular or mycelial growth occurs through the operation of primary metabolism, and a later "idiophase," when growth slows down or ceases and when secondary metabolic pathways start to operate.

Regulation of Secondary Metabolic Pathways

Understanding of the genetic basis of the regulation of primary metabolic pathways is most advanced in certain eubacteria, notably *E. coli*. Rather precise models describe specific controls for transcription of particular operons or for the activity of particular enzymes as well as for more generalized controls operating through catabolite repression via the concentrations of effector molecules such as cyclic nucleotides (245). The study of isolated mutations was the single most powerful tool needed to describe such control systems. In fungi much less is known, but comparable mechanisms involving specific regulator genes and generalized control (through carbon or nitrogen catabolite repression) are being revealed by the study of mutants (62, 302).

For antibiotics, evidence is accumulating for induction or derepression, at the onset of idiophase, of genes specifying the biosynthetic en-

zymes, and attempts are being made to recognize those effector molecules or signals that are directly involved, whether these include inorganic phosphate, adenosine 5'-triphosphate, energy charge, cyclic nucleotides, or others (252, 298). However, it is difficult to see how such control systems can be understood without the isolation of mutations specifically modifying or abolishing them. A start has been made in the study of the regulation of penicillin and cephalosporin synthesis (see below), but much more genetic analysis of regulatory mutants is needed, particularly a study of their dominance relations, etc., in order to build satisfying models of regulation.

Pleiotropic Effects of Mutations on Antibiotic Production

In any mutational study of antibiotic-producing organisms, mutations are encountered that reduce or abolish production but whose effect is reasonably interpreted as indirect and nonspecific.

To carry out a genetic analysis of antibiotic production, genetic markers are required. Auxotrophic mutations are often used, and these commonly depress titer significantly. Bonner (33) found only 4% of 400 monoauxotrophs of *P. notatum* defective in penicillin production, but these results appear to be rather atypical. Macdonald et al. (189) found the majority of amino acid and vitamin auxotrophs of *P. chrysogenum* to have yields less than half that of their parents. In *Emericellopsis* species, auxotrophic markers usually caused a significant reduction in or complete loss of antibiotic production (87). Pleiotropic depression of antibiotic titer by auxotrophic mutations seems also to be common in streptomycetes (81, 214).

Morphological mutations, also, tend to have pleoitropic effects on antibiotic production. In *B. subtilis*, 20% of sporulation mutants showed no antibiotic activity (19), and all these were blocked at stage 0, suggesting an association between antibiotic production and an early step in sporulation. In *S. coelicolor*, production of actinorhodin and methylenomycin is prevented by mutation at the *bldA*, *B*, and *D* loci, all of which prevent normal aerial mycelium development (211); on the other hand, the variants of *Streptomyces alboniger* that had lost the ability to produce aerial mycelium, and which may have arisen by plasmid loss, still produced puromycin (243).

One of the most detailed studies of a relationship between antibiotic synthesis and morphological differentiation concerns *C. acremonium* (221). In submerged culture, four morphological forms occur: hyphae, arthrospores, conidia, and germlings. The phase of hyphal differentiation into arthrospores coincides with the maximum rate of β-lactam antibiotic synthesis (272), and, when arthrospores were enriched by density centrifugation, they were found to have 40% greater antibiotic-producing activity than any other morphological cell type (221). Furthermore, in a series of mutants, each with an increased potential to produce β-lactam antibiotics, differentiation into arthrospores was directly proportional to the increased antibiotic titer (221). Since a nonantibiotic-producing mutant readily differentiated into arthrospores, antibiotic synthesis was not a prerequisite for cellular differentiation; this is the case also in *S. coelicolor*, in which most nonproducing mutants of actinorhodin and methylenomycin differentiate normally, and in *B. licheniformis*, in which a bacitracin-negative mutant sporulated normally (117).

The Adaptative Significance of Antibiotics

There has been much speculation on this topic, and diametrically opposed conclusions have been reached (71). That antibiotics are in fact produced in nature, albeit in low concentrations, seems beyond doubt (305). That their production is adaptive, at least in the majority of cases, is a priori almost certain in view of the difficulty of finding adaptively neutral characters in serious studies of population genetics of higher organisms. Increased knowledge of the genetic control of antibiotic production could undoubtedly illuminate discussions of their natural role. For example, the finding (210) that the majority wild strains of *A. nidulans* produced a similar titer of penicillin but that the titer-demonstrating genes were different in different strains provides strong support for the adaptive nature, not only of the capacity to produce an antibiotic, but of a particular level of antibiotic synthesis.

"Low Specificity" of Secondary Metabolic Enzymes

The typical result of mutational loss of an enzyme of a primary biosynthetic pathway is interruption of the pathway with accumulation of the intermediary metabolite immediately before the block; rather rarely is this metabolized by a side pathway to a "shunt" product. This follows from the fact that most pathways of primary metabolism are catalyzed by enzymes of high specificity for their normal substrate. A series of modifications to a carbon skeleton, for example, must occur in a precise sequence if they are to take place. In contrast, enzymes catalyzing steps of secondary metabolism are much less sensitive to variations in the substit-

uent functions of a molecule. This means that not all traffic along a pathway is going by the same route from starting material to end product; the different functions may be carried out in various sequences so that the system resembles a metabolic "grid" rather than a linear pathway (43, 44). In wild-type organisms, various combinations of the functions may occur to give a "family" or "complex" of related antibiotic molecules, whereas in mutants lacking a pathway enzyme, simple accumulation of an intermediate will not always occur; often enzymes normally functioning later in the sequence will still operate with the production of a shunt metabolite. One of the best examples is in the tetracycline pathway (see below). These factors complicate deductions about biosynthetic pathways made from the nature of mutant metabolites or from the pattern of cosynthesis (the secretion of an antibiotic precursor by a blocked mutant that can be converted to antibiotic by a mutant blocked at an earlier step in the pathway) between pairs of blocked mutants. On the other hand, they greatly widen the scope for the production of unnatural antibiotics by mutational biosynthesis (see below).

Chemical Classes of Antibiotics

At first sight, antibiotics, particularly those produced by actinomycetes, have a bewildering array of chemical structures. However, biochemical studies indicate considerably more similarity in their biosynthetic routes than the diversity of their structures might suggest. A particularly important unifying hypothesis is the polyketide concept (30, 286). The idea is of a close analogy with fatty acid synthesis in which a multienzyme "fatty acid synthetase" catalyzes the sequential addition, associated with decarboxylation, of 2-carbon units from malonyl coenzyme A (CoA) "extender" units to a 2-carbon acetyl CoA "primer," the growing carbon chain remaining enzyme bound until fully grown. In the case of some actinomycete antibiotics, the primer is propionyl CoA and the extender is methylmalonyl CoA, so that 3-carbon units are added, leading to the presence of methyl groups attached to the growing polyketide chain; various other variations are also possible. By cyclization reactions, the polyketide is stabilized, and only at this stage are free biosynthetic intermediates of the final antibiotic produced. Enzymological studies appear to be most advanced for 6-methylsalicylic acid synthetase from *P. patulum* (75), which is involved in the synthesis of the antibiotic patulin, but evidence for the existence of multienzyme polyketide synthetases, susceptible, like fatty acid synthetases, to inhibition by the antibiotic cerulenin (228), is available for several of the important actinomycete antibiotics, including tetracyclines, erythromycins, and rifamycins (see below). Not all antibiotics, of course, arise in this way. Many are put together from moieties derived from typical primary metabolites such as amino acids or sugars: the β-lactam antibiotics characteristic of fungi and the large class of aminocyclitol actinomycete antibiotics are examples. Still another completely different biosynthetic mode is the nonribosomal peptide assembly process characteristic of many antibiotics of bacilli.

Why "Biogenesis"?

Many biochemical papers refer to the biosynthesis of antibiotics as "biogenesis." This seems to be an unnecessary term with almost mystic overtones. A more serious criticism applies to its adjectival form "biogenetic," which implies that studies described by this term have a genetic content. What is wrong with the terms biosynthesis and biosynthetic?

MUTATIONAL STUDIES OF ANTIBIOTIC SYNTHESIS

Fungi

P. chrysogenum. In 1947 Bonner (33), noting that 25% of lysine auxotrophs produced no penicillin, predicted a common precursor of penicillin and lysine. Ten years later (68), lysine was found to be a potent inhibitor of penicillin synthesis; a precursor of lysine biosynthesis in fungi, α-aminoadipic acid, not only reversed the inhibition due to lysine but stimulated penicillin synthesis in its absence (273). In the meantime, a noncyclic tripeptide containing residues of α-aminoadipic acid, cysteine, and valine was isolated (14), giving rise to the tripeptide theory of penicillin biosynthesis.

Genetic evidence for α-aminoadipic acid as an essential intermediate in penicillin synthesis was obtained (111, 220). Lysine auxotrophs fell into two phenotypic classes with regard to penicillin synthesis: mutants blocked before α-aminoadipic acid could make penicillin in the presence of lysine only if α-aminoadipic acid was added, but those blocked after α-aminoadipic acid synthesized antibiotic when grown with lysine alone. Lysine exerts end product control on α-aminoadipic acid formation, and, hence, decreases the amount available for penicillin biosynthesis (111). These results and labeling experiments appear to confirm that both α-aminoadipic acid and the tripeptide L-(α-aminoadipyl)-L-cysteinyl-D-valine are penicillin

precursors (89), but the final stages of the pathway are unknown.

Two possible precursors of benzylpenicillin (penicillin G) have been isolated from fermentations devoid of side chain precursors: isopenicillin N (94) and the penicillin nucleus, 6-APA (24). Most evidence favors isopenicillin N as the precursor of the penicillins produced by *P. chrysogenum* (70), and 6-APA may be a shunt metabolite derived by deacylation of isopenicillin N in the absence of a side chain precursor. The terminal reaction of penicillin G synthesis is therefore likely to be an exchange of the L-aminoadipic acid side chain of isopenicillin N for phenylacetic acid from phenylacetyl CoA. An acyltransferase that could catalyze such a reaction occurs in crude extracts, and this enzyme or multienzyme complex may catalyze at least five other related reactions (70). The postulated pathway of penicillin G synthesis is shown in Fig. 1.

In spite of the attention paid to the parasexual cycle in *P. chrysogenum*, it is remarkable that very little use has been made of blocked mutants to study penicillin biosynthesis. Some early studies of such *npe* mutants were described by Sermonti (48, 260). A more detailed complementation analysis was reported by Holt et al. (129). A sample of mutants producing less than 10% of the parental titer were selected from strain NRRL 1951. Five complementation groups (V, W, X, Y, and Z) were identified with 1, 2, 2, 9, and 1 representatives, respectively (187). Mutants in four of the five groups were morphologically normal, but the group Z mutant had a reduced growth rate in surface culture. Parasexual analysis assigned members of groups W, X, Y, and Z to the same haploidization group (chromosome) and the group V mutant to a separate chromosome.

Preliminary characterization of a number of *npe* mutants (220) used a lysine auxotroph blocked after α-aminoadipic acid. This strain synthesized penicillin when grown in the presence of lysine; when ^{14}C-labeled α-aminoadipic acid was supplied, label was incorporated into seven intracellular compounds including α-aminoadipyl-cysteinyl-valine and isopenicillin N. Eleven *npe* mutants selected from this auxotroph were examined for their ability to form the labeled intermediates. A complex pattern was found, and the eleven mutants could be grouped into nine types. Only one compound was common to all the mutants, and the results did not unambiguously suggest an order for biosynthesis of the seven compounds. Analysis of the biosynthetic pathway may, however, have been confounded by the production of shunt metabolites as has been found for cephalosporin nonproducing mutants (see below).

Regulation of penicillin biosynthesis. Regulation of three primary pathways, for lysine, cysteine, and valine, influences directly the synthesis of penicillin. Regulation of these

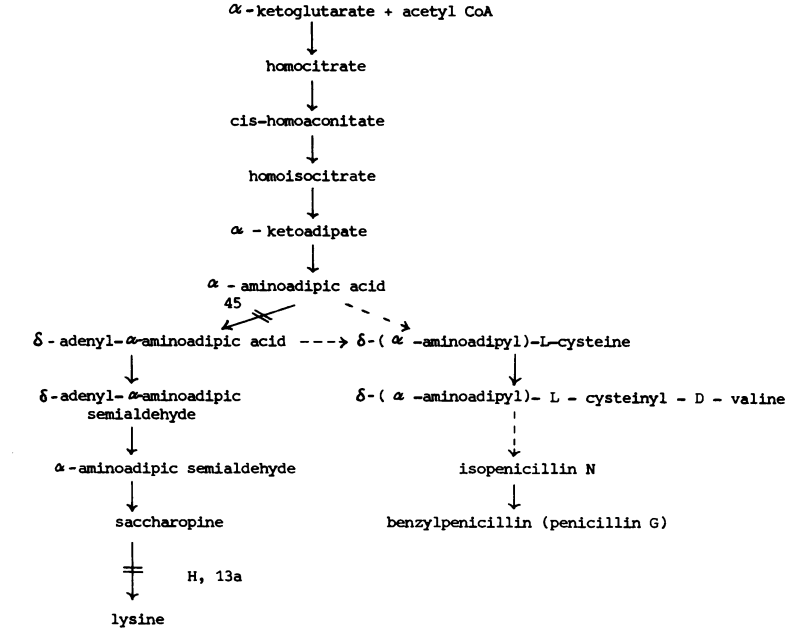

FIG. 1. *Postulated pathway for benzylpenicillin synthesis (72). The blocks in the three lysine auxotrophs (H, 13a, and 45) described by O'Sullivan and Pirt (229) are indicated.*

pathways has been studied in two ways: by selecting known types of mutation in the pathway and characterizing their effects on antibiotic production; or by comparing strains selected for increased titer with their parent strain and determining whether or not alterations had been selected in the primary pathways.

Demain (69) postulated a branched pathway for lysine and penicillin (Fig. 1) and suggested that the inhibitory effect of lysine on penicillin biosynthesis was due to negative feedback control of an early enzyme. Lysine feedback inhibits homocitrate synthase, an early enzyme of lysine biosynthesis, in yeast (195) and in *Neurospora crassa* (125). A similar inhibition was demonstrated in *P. chrysogenum* by Demain and Masurekar (73), who isolated nine lysine auxotrophs of the Wis54-1255 strain, one of which was a leaky mutant that could be satisfied by α-aminoadipic acid. It accumulated homocitrate, and this accumulation was inhibited 50% by 0.3 mM lysine. Such feedback inhibition by lysine occurred in both the growth and the penicillin production phases and suggests that penicillin synthesis could be extremely sensitive to lysine present during production. Homocitrate synthase activity in crude extracts of the early lysine auxotroph was found, somewhat surprisingly, to be insensitive to lysine inhibition (202) for unknown reasons.

Inhibition of penicillin production under fermentation conditions requires higher exogenous concentrations (10 to 20 mM) of lysine than those in a commercial medium. However, endogenous production of lysine might significantly limit penicillin production. This possibility was examined by selecting lysine-overproducing mutants by their resistance to a lysine analog; they showed significantly reduced penicillin formation (201). It was suggested that these mutants were derepressed at a point after the branch point in the lysine pathway, resulting in overproduction of lysine and so an underproduction of penicillin. These results indicate an intimate intracellular relationship between the biosynthesis of lysine and penicillin and suggest that mutants desensitized to lysine inhibition of homocitrate synthase might be superior producers of penicillin.

The apparent regulatory control of penicillin production by lysine led Pirt (234) to postulate that if a lysine auxotroph blocked after α-aminoadipate were fed lysine at a low level, then the feedback control could be overcome and penicillin production increased. Three lysine auxotrophs of Wis54-1255 blocked after α-aminoadipic acid were selected (229); two (H and 13a) were blocked between saccharopine and lysine and showed very low saccharopine reductase activity, and the other (45) showed no α-aminoadipic acid reductase activity (Fig. 1). All these blocks caused a large decrease in penicillin synthesis, and only in strain H could this be overcome by feeding lysine. However, maximum penicillin production by strain H required lysine feed concentrations 180% in excess of that required for growth, and even then the titer was less then that of the parent with no lysine feed. The virtually complete failure of strain 45 (blocked in α-aminoadipic acid reductase) to produce penicillin suggested that the branch point between lysine and penicillin pathways may be adenyl α-aminoadipic acid rather than α-aminoadipic acid (Fig. 1).

A second aspect of primary metabolism influencing penicillin synthesis is sulfur metabolism. Studies with ^{35}S showed that sulfur for penicillin comes efficiently from sulfate via the sulfate reduction pathway but can also be derived by reverse transsulfuration from methionine. Tardrew and Johnson (281) examined the uptake of inorganic sulfate and the excretion of organic sulfate during the penicillin-producing phase in a number of wild-type and improved strains. The high-yielding mutants took up more inorganic sulfur from the medium than the wild type, and the concentration of inorganic sulfur in the mycelium of one mutant (Wis51-20F3) was at least twice that in its ancestral strain NRRL 1951-B25. This increased capacity for sulfur uptake was apparently introduced between strain X1612 and strain Q176, which was obtained from X1612 by UV irradiation, and it may be that this change in metabolism is primarily responsible for the doubling in titer between X1612 and Q176.

The third amino acid involved in the biosynthesis of penicillin is valine, which is synthesised by the same pathway in fungi and bacteria. In *P. chrysogenum* Q176, the first enzyme in the valine pathway, acetohydroxy acid synthetase, is sensitive to feedback inhibition by valine (112). In a high-titer derivative of Q176, the enzyme had lost one of the two binding sites for valine, allowing increased formation of the amino acid. The high-titer strain also produced more enzyme than Q176.

A. nidulans. Although it is not used commercially, the availability of sexual and parasexual cycles in *Aspergillus nidulans* and its extensive use in genetic studies (155, 237, 238) have prompted several workers to use it as a model for genetic analysis of penicillin production.

Mutants were classified as "penicillinless" (*npe*) if they had a titer of 10 to 0% of the

parental level (129). Twenty-eight such mutants were selected after treatment with a variety of mutagens; 11 had a significantly lower colony radial growth rate than the parent strain, and 8 showed changes in mycelial or conidial pigmentation. In at least one (*npe* C007), such effects appeared to be due to pleiotropy of the *npe* mutation. A heterokaryon test (151) between *npe*$^+$ and *npe*$^-$ strains showed that the *npe* character was under nuclear control and all the mutations were recessive in diploids. The 28 mutations fell into at least five complementation groups, one of them (*npeA*) containing 20 mutations (187). The *npeA* locus was mapped by parasexual and sexual analysis (129) to chromosome VI, probably distal to *sbA*. As in *P. chrysogenum*, biochemical analysis of these mutants will be of considerable use in elucidating the pathway and control of penicillin biosynthesis, while application of genetics should be much more straightforward.

C. acremonium. *Cephalosporium* (and *Emericellopsis*) species produce two β-lactam heterocyclic antibiotics with D-α-aminoadipic acid as an acyl side chain and either 6-APA (PCN) or 7-ACA (CPC) as nuclei. Little or no free 6-APA and no 7-ACA is formed during a *Cephalosporium* fermentation, and the addition of side chain precursors has no influence on the antibiotics formed, which are always PCN or CPC.

Cephalosporia produce a tripeptide [L-(α-aminoadipyl)-L-cysteinyl-D-valine] similar to that produced by penicillia, although the intracellular levels are much lower. Two tetrapeptides containing α-aminoadipic acid, cysteine, valine, and glycine or α-aminoadipic acid, cysteine, β-hydroxyvaline, and glycine have also been isolated from mycelia of *C. acremonium* (181), but their involvement in CPC and PCN biosynthesis is not understood.

Biosynthesis of the tripeptide. Genetic studies have clearly indicated involvement of the tripeptide in antibiotic synthesis. Lemke and Nash (173a) obtained seven mutants that could not synthesize β-lactam antibiotics. Six of them were prototrophic and fell into two classes. Two mutants (peptide$^-$) did not incorporate label from valine or α-aminoadipic acid into the cysteine-containing peptides, whereas the remaining four (peptide$^+$) did so. Mutants of the first class were presumably blocked in the synthesis of the peptides, and those of the second class were blocked in the conversion of the peptides into antibiotics. Mutants of these same two phenotypic classes were also isolated by others (241; T. Kanzaki, personal communication). Pairs of inactive mutants did not cosynthesize antibiotics when grown in mixed culture, but a heterokaryon between two peptide$^-$ mutants, although rather unstable, synthesized antibiotic at 10% of the parental level (220). This observation is of particular interest since it suggests that at least two genes may be involved in the synthesis of the tripeptide from its constituent amino acids, although no intermediates have been identified. Heterokaryons between peptide$^-$ and peptide$^+$ mutants produce antibiotic (S. Queener and T. Kanzaki, personal communications), and in one heterokaryon between two peptide$^+$ mutants no antibiotic was produced (Kanzaki, personal communication).

The seventh mutant isolated by Lemke and Nash (173a) was a lysine auxotroph blocked in a step after α-aminoadipic acid. The mutant synthesized trace amounts of antibiotic- and cysteine-containing peptides in the presence of α-aminoadipic acid and lysine but not with lysine alone. This result is surprising since one would expect such a mutant to produce PCN and CPC when grown on lysine alone. Essentially similar results were reported by Nuesch et al. (Abstr. 4th Int. Ferm. Symp., Kyoto 1972, p. 228) for lysine auxotrophs blocked before or after α-aminoadipic acid. The lack of antibiotic production is apparently due to extreme sensitivity of the mutant to lysine feedback inhibition (70).

Biosynthesis of PCN. Virtually nothing is known about subsequent reactions in penicillin N (PCN) synthesis, but it seems unlikely that 6-APA is involved since only trace amounts are found in mycelium grown on complex media. Lemke and Nash (173a) obtained a mutant with increased ability to produce 6-APA; it produced penicillin N but no cephalosporin C.

Biosynthesis of CPC. *Cephalosporium* species produce two major compounds in addition to CPC, deacetylcephalosporin C (DCPC) and deacetoxycephalosporin C (DOCPC) (104, 122). The biosynthetic relationships between these compounds have been demonstrated very clearly by the use of blocked mutants (CPC$^-$).

All CPC$^-$ mutants (102, 104, 105, 179, 241) fall into three classes, accumulating PCN, DOCPC, and DCPC; PCN and DOCPC; or PCN only. This is consistent with a linear biosynthetic pathway: PCN → DOCPC → CPC. An alternative branched pathway (Fig. 2) with PCN and CPC as end products (102, 241) is also consistent with the observed accumulation patterns but predicts the existence of mutants (CPC$^+$ PCN$^-$) producing CPC but not PCN, and no such mutants have been reported. A test for labeling of CPC by [^{14}C]PCN (272) failed because the penicillin was not taken up by the

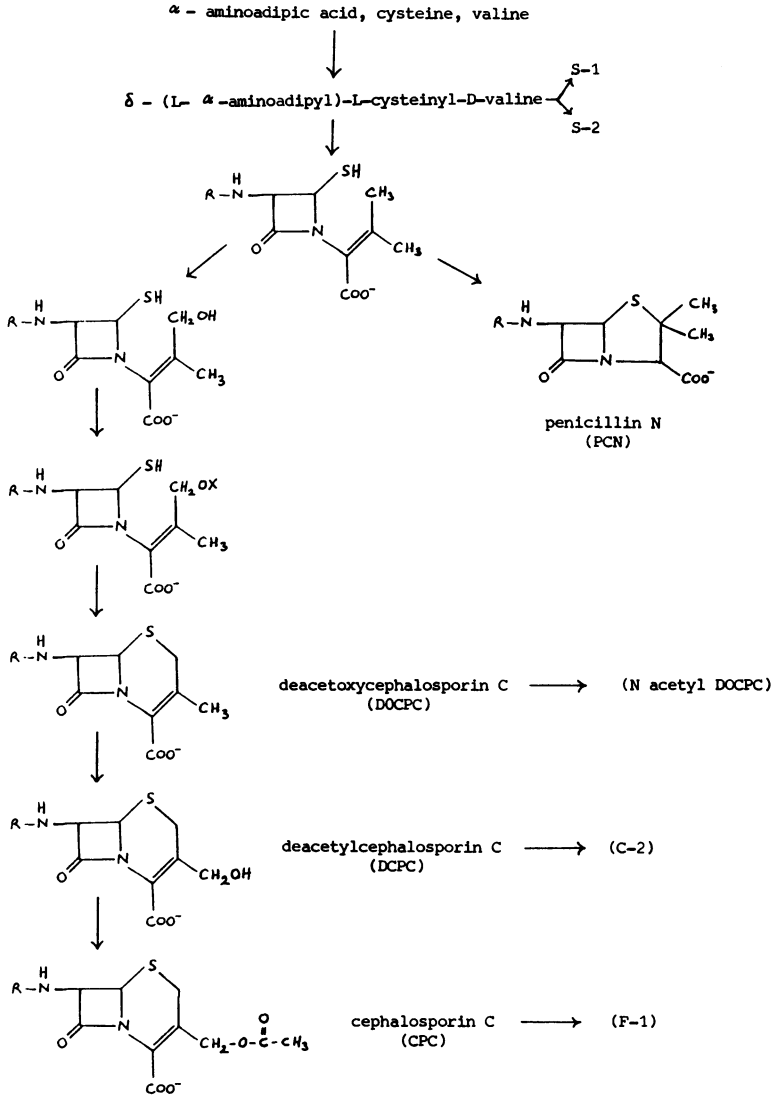

FIG. 2. *Hypothetical branched pathway for biosynthesis of PCN and CPC (244) and suggested shunt metabolites (159, 285).*

cells. Recently, however, cephalosporin synthesis by cell-free extracts of *C. acremonium* was claimed to be markedly stimulated by PCN but not by penicillin G or 6-APA (166). These results, taken with the absence of CPC^+ PCN^- mutants, suggest that PCN may indeed be an intermediate in cephalosporin synthesis.

Further evidence for the final step of the proposed pathway was provided by the demonstration of an acetyl CoA:DCPC acetyl transferase that converts DCPC to CPC in CPC^+ strains (104, 179) but was lacking in four out of five CPC^- mutants (104), thus accounting for DCPC accumulation. An enzyme catalyzing the hydroxylation of DOCPC to DCPC has also been reported (102, 179).

Several apparent shunt metabolites have also been found in CPC^- mutants (Fig. 2). Those blocked before DOCPC, some of which also produced no PCN, accumulated the dimer of L-(α-aminoadipyl)-L-cysteinyl-D-valine and the disulfide of the tripeptide and methanethiol (159). Some of the mutants accumulating DOCPC and PCN produced traces of CPC and accumulated a new compound identified as *N*-acetyl DOCPC (285). Mutants accumulating DCPC also produced a second compound (C-2) believed to be derived from DCPC (101), and in

a potent CPC-producing mutant a new compound (F-1) was identified, apparently derived from CPC (160). Hence, CPC provides another clear example of the accumulation of shunt metabolites in mutants blocked in the biosynthetic pathway of a secondary metabolite.

Regulation of CPC biosynthesis. CPC and PCN biosynthesis is substantially stimulated by methionine, especially the D-isomer, but the exact mechanism is uncertain. Methionine donates sulfur efficiently to CPC (50, 223), and sulfur incorporation is thought to occur via the reverse transsulfuration pathway (Fig. 3). Whether or not this is the only role for methionine has, however, been questioned for a number of reasons. First, the postulated intermediates between methionine and CPC (homocysteine, cystathionine, and cysteine) will not replace methionine (74, 223). Second, the nonsulfur analog of methionine, norleucine, will replace methionine (78). Third, prototrophic strains produce moderate levels of CPC in a defined medium with sulfate as sole surface source. Drew and Demain (80) asked whether such antibiotic formation involved methionine, or whether it was due to passage of sulfur directly from sulfate via cysteine to CPC (Fig. 3). The introduction of a mutation in the transsulfuration pathway between cystathionine and homocysteine eliminated antibiotic production, even in the presence of excess sulfate, thus implying an obligatory role of endogenous methionine in the regulation of cephalosporin biosynthesis.

This conclusion was reinforced by the isolation of two further mutants (79). A mutant (274-1) blocked between sulfate and cysteine (Fig. 3) grew well on both methionine and cysteine, but antibiotic production on cysteine was only 30% of that on methionine. Thus, even in the absence of any possible competition by inorganic sulfate, only methionine was effective in stimulating antibiotic synthesis. A double mutant (11-8) blocked not only in the pathway from sulfate to cysteine but also in the pathway from cysteine to methionine (Fig. 3) produced virtually no CPC when grown in excess cysteine (plus a low level of methionine necessary to support growth), but supplementation by either methionine or norleucine very efficiently stimulated cephalosporin synthesis. Such genetic and biochemical analysis has therefore provided strong evidence that methionine stimulation of CPC production is due to a role of methionine other than that of sulfur donation, but there is as yet little indication of what this role may be.

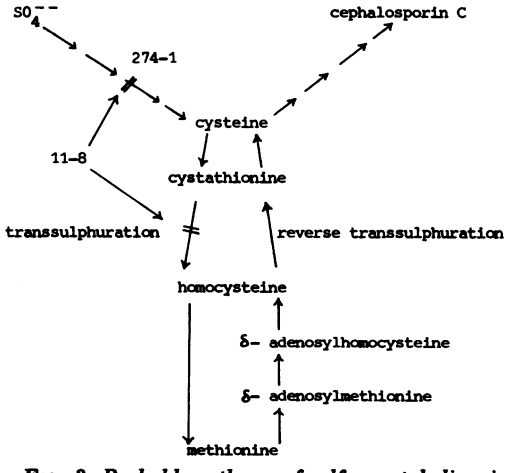

FIG. 3. *Probable pathway of sulfur metabolism in Cephalosporium acremonium (79) showing positions of blocks introduced by mutation in strains 274-1 and 11-8.*

The improvement of CPC production by strain selection has undoubtedly been due, in part, to selection of regulatory mutations both in the CPC pathway and in related primary pathways. Queener et al. (242) showed that in a number of high-yielding mutants of *C. acremonium* M8650 the level of glutamate dehydrogenase activity is four or five times higher than in the parent strain, and it seems likely that altered regulation of glutamate dehydrogenase synthesis has increased the supply of L-glutamic acid and hence of α-amino nitrogen for the three constituent amino acids of CPC.

In the fifth of the CPC^- mutants isolated by Fujisawa et al. (104; see above), CPC was synthesized and then enzymatically hydrolyzed to DCPC by an extracellular CPC acetylhydrolase (CAH) that was not detected in the parent strain or in the other four mutants (103). A similar CAH activity was demonstrated by A. Hinnen and J. Nüesch (personal communication) in a high-titer derivative of M8650. This activity appeared after 120 h and was paralleled by exhaustion of glucose. They suggested that synthesis of CAH may be regulated by carbon catabolite repression, and they also reported that mutants with highly reduced CAH activity can be selected that then retain a high titer of CPC under conditions of glucose starvation.

P. griseofulvum. The only description of the use of an induced mutation to alter the antibiotic produced by a fungus concerns the production of the 2'-ethoxy analog of griseofulvin (150). Griseofulvin contains three methyl groups derived from choline, and, on the basis of work with tetracyclines (82), it was anticipated that ethoxy analogs of griseofulvin could

be produced under appropriate fermentation conditions. Six methionine auxotrophs of a strain of *Penicillium griseofulvum* were obtained, three of which grew to a varied extent with cystine and three of which could utilize sodium thiosulfate. Ethionine was highly toxic to the parent strain of *P. griseofulvum* and to one of the auxotrophs. Four other auxotrophs showed reduced growth on ethionine (200 μg/ml), and one was resistant. Three of the auxotrophs were shown to produce a new antibiotic, the 2'-ethoxy analog of griseofulvin, when grown in ethionine-supplemented fermentation broths. This compound had been considered as having possible agricultural application (65), but it was abandoned because of poor translocation in plants.

P. patulum. *P. patulum* produces the antibiotic patulin as an extracellular end product via the classical aromatic polyketide intermediate, 6-methylsalicylic acid (93, 216). The major pathway of patulin biosynthesis in *P. patulum* (synonymous with *Penicillium urticae* and *Penicillium flexuosum* and closely related to *P. griseofulvum*) is part of a proposed matrix of reactions involving up to 16 metabolites (44) and is one of the most extensively studied secondary metabolic pathways in fungi. Although patulin is of no commercial interest, owing to its high toxicity to animals (271), the presence of a parasexual cycle in *P. patulum* (49) and the extensive biochemical studies available make genetic analysis of this system an attractive proposition as a model secondary metabolic matrix.

O. mucida. This basidiomycete, *Oudemansiella mucida,* produces an antifungal antibiotic, mucidin (217). Nonproducing mutants were isolated after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and genetic analysis indicated that each of the mutants differed from the producing monokaryons in a single gene (M. Semerdžieva, personal communication).

Actinomycetes

Limited biosynthetic studies of many streptomycete antibiotics have been made, and in many of the strains some antibiotic-nonproducing mutants were probably isolated. However, in few cases have sufficiently large series of mutants been studied to make a crucial contribution to the clarification of biosynthetic pathways, and in even fewer cases have the mutants been analyzed genetically. We shall review those cases where blocked mutants have been important for deducing steps in the biosynthesis of important antibiotics or where recombination studies have been made.

Tetracyclines. There are two main producers of tetracycline antibiotics: *Streptomyces aureofaciens,* wild types of which make mainly chlortetracycline, and *Streptomyces rimosus,* the producer of oxytetracycline. Mutants of *S. aureofaciens* failing to chlorinate the molecule in position 7 accumulate tetracycline. Oxytetracycline differs from tetracycline by hydroxylation at position 5.

The similarity between the antibiotics of the two strains suggests a largely common biosynthetic pathway, so that a study of gene-enzyme relationships for one would probably provide a good model for both. However, biochemical studies, aided by a rather complete set of blocked mutants, are most advanced in *S. aureofaciens,* in which recombination studies are little developed, whereas in *S. rimosus* genetic studies have not been complemented by biochemical characterization of the mutants.

Tetracycline biosynthesis provides a good example of the polyketide route. McCormick (206) presented a pathway of chlortetracycline synthesis by *S. aureofaciens,* the early steps somewhat speculative, in which one molecule of malonamyl CoA and eight of malonyl CoA are condensed to a nonaketide that is cyclized to give a ring system for subsequent substitution at specific positions. A complete set of blocked mutants for the reactions involved in the polynuclear part of the pathway was crucial in defining the steps. These mutants were used in pairwise cosynthesis tests, and the precursors, or more often shunt products, that they accumulated could be identified. Extensive biosynthetic studies were also made by the Prague group (147), who have emphasized particularly the series of up to 72 compounds (the shunt products of McCormick) that could arise when each of 11 biosynthetic steps, after the formation of a hypothetical tricyclic nonaketide, is blocked.

Recombination was first described in *S. aureofaciens* by Alikhanian and Borisova (9) and has been studied more recently by the Prague group (32). Unfortunately, no linkage map is available, and the locations of the pathway genes for chlortetracycline are unknown, although some clustering was suggested by a failure to obtain antibiotic-producing recombinants by selecting prototrophs from crosses of auxotrophs carrying mutations blocking the pathway at different steps.

The genetics of *S. rimosus* is much further advanced, but genetic determination of the biosynthetic pathway to oxytetracycline is still incompletely known. No data on the mapping of blocked mutants in the strain studied by Friend and Hopwood (96) have yet been published. In

the strain studied in Zagreb, blocked mutants belonging to at least four cosynthesis groups were mapped to one region of the circular linkage map (5), but no results of fine mapping have been reported. In a third strain, mutants belonging to nine cosynthesis groups were mapped in two clusters (35), but, in the absence of a general linkage map of the strain, the relative spacing of these two groups was unknown. They fell between two streptomycin resistance loci, but the argument that these corresponded to *strA* and *strB* in *S. coelicolor* A3(2), and were therefore fairly close, needs confirmation. We can probably conclude from all these studies that there is significant clustering of the pathway genes for tetracyclines on the chromosomes of the producing organisms, but there is no information on the precise spacing of the genes, nor any details of gene-enzyme relationships. The possible involvement of a plasmid in oxytetracycline production is discussed below.

Macrolides. *Erythromycins.* There are four main erythromycins, diglycosides of 14-membered lactones (59, 194). All four have the same basic sugar, desosamine; they differ in the neutral sugar and at position 12 in the lactone ring. Erythromycins A (the clinically important compound) and C have a 12-hydroxyl, whereas B and the recently discovered D do not; the neutral sugar is cladinose in A and B, and mycarose (lacking a methylether of cladinose) in C and D.

The lactone skeleton of erythromycins is apparently derived from a polyketide chain synthesized from propionate units (291) by successive condensation of six methylmalonyl CoA units onto a propionyl CoA primer, and the properties of a multienzyme "lactone synthetase" involved are being studied. The lactone so formed is apparently 6-deoxyerythronolide B, which is hydroxylated at position 6 to yield the lactone found in erythromycins B and D (erythronolide B). Attachment of mycarose and, subsequently, desosamine to the lactone would yield erythromycin D, postulated to be the parent member of the erythromycin family. This can suffer two substitutions in either order. *O*-methylation of the mycarose of erythromycin D by a *S*-adenosyl methionine-dependent transmethylase would yield B, and hydroxylation of its lactone would then give A. Alternatively, hydroxylation of the lactone of D would give C followed by *O*-methylation of mycarose to give A (194).

Biochemical studies of blocked mutants were instrumental in deducing certain features of this scheme, notably that 6-deoxyerythronolide B is a precursor of erythronolide B (198) and that mycarose is then added to this lactone to yield a neutral monoglycoside (199). A presumed earlier blocked mutant accumulated a lactone that appeared to be a shunt compound, possibly arising through lack of a gene product forming part of the presumed multienzyme lactone-synthesizing complex (197). A further use of blocked mutants was the identification of a fifth erythromycin, E, formed when blocked mutants unable to make the erythromycins were fed with erythromycin A (196). E is apparently derived from A by a change in the linkage of the cladinose to the lactone ring. Although recombination has twice been reported in *Streptomyces erythreus* (67, 162), it has not been extended to linkage analysis, and blocked mutants have not been analyzed genetically. Information on the location of biosynthetic genes is therefore completely lacking.

Platenomycins. Twenty-four blocked mutants of *Streptomyces platensis* subspecies *malvinus*, the producer of these basic 16-membered macrolides, were classified into seven cosynthesis groups; one group contained non-consynthesizing mutants, and two contained mutants that acted only as secretors (106). These two classes were found to secrete, respectively, monoglycosides, lacking the mycarose moiety of the platenomycins, and lactones (platenolides), lacking both this sugar and the mycaminose moiety. The terminal part of the platenomycin synthesis pathway was, therefore, proposed (107) to proceed from the platenolides by successive addition of the two sugar moieties, which form a disaccharide linked at one point to the lactone instead of being independently attached to it as in erythromycin. No studies have been reported on other classes of blocked mutants, and no genetic analysis has been described; thus the locations of the genes concerned are unknown.

Turimycin. Possible plasmid involvement in the synthesis of this macrolide by *Streptomyces hygroscopicus* is discussed below.

Aminocyclitols (aminoglycosides). Compounds of this class consist of two to five moieties joined by glycosidic linkages; one moiety is an aminocyclitol (cyclohexane with amino and alcohol substitutions), and the others are sugars. In most commercial members of the class (neomycin, kanamycin, paromomycin, ribostamycin, tobramycin, destomycin, hygromycin B, lividomycin, validamycin, gentamicin, sisomicin), the aminocyclitol is deoxystreptamine, but in streptomycin it is streptidine, in spectinomycin, actinamine, and in bluensomycin, bluensidine. There is a recent comprehensive review of the biosynthesis of the group (246) as well as earlier reviews of streptomycin

biosynthesis (72, 295, 296).

The vast majority of biosynthetic studies have used the approach of feeding radioactively labeled or ^{13}C-enriched precursors to biosynthetically proficient strains, and there have also been some enzymatic studies. With varying degrees of certainty, the various aminocyclitol and sugar moieties have been shown to be derived from glucose, and a few of the pathways have been deduced in considerable detail, notably for streptidine in *S. griseus*. Details of the linking of the various moieties are few; probably dihydrostreptose is added to streptidine-6-phosphate to form a disaccharide intermediate in streptomycin synthesis (165).

Blocked mutants have contributed extremely little to pathway clarification, since significant series of such mutants do not appear to have been isolated. Study of a mannosidostreptomycin-negative mutant of *S. griseus* indicated that mannosidostreptomycin is not an essential intermediate in streptomycin synthesis (72), and a few tentative conclusions on the joining sequence of some antibiotic moieties have been deduced by feeding blocked mutants with individual moieties or linked pairs or trios of them (246, 256).

Although recombination was reported early in *S. griseus* (streptomycin) and *Streptomyces fradiae* (neomycin) (40), no linkage studies have been made nor have crosses involving blocked mutants been described. The same is true of recombination studies in strains of *Micromonospora echinospora* and *M. purpurea* making gentamicins (28). In fact, there appears to be no published information about the location of any gene concerned with the biosynthesis of any aminocyclitol antibiotic.

Mutational biosynthesis. The aminocyclitol antibiotics, built from separate moieties, lend themselves to a technique first described by Schier et al. (255) and later designated "mutational biosynthesis" (218). Mutants blocked in the synthesis of one moiety (in this case the aminocyclitol) are fed with analogs of that moiety, which are incorporated by the mutant into unnatural antibiotics. For *S. fradiae*, *Streptomyces rimosus* forma *paromomycinus*, *Streptomyces kanamyceticus*, *Streptomyces ribosidificus*, and *Micromonospora inyoensis*, making neomycin, paromomycin, kanamycin, ribostamycin, and sisomicin, respectively, mutants blocked in deoxystreptamine biosynthesis were used (167, 254, 255, 283); for *S. griseus*, making streptomycin, streptidine-negative mutants were fed (218). The analogs used, with varying degrees of success, were streptamine, epistreptamine, and several others.

Novobiocin. This antibiotic consists of three moieties, rings A, B, and C, which are a substituted benzoic acid, a substituted aminocoumarin, and a substituted sugar, carbamoylnoviose, respectively. Blocked mutants have so far made only a modest contribution to explaining the pathway of novobiocin synthesis by *Streptomyces niveus*. Mutants confirmed the roles of carbamylation and methylation of the noviose sugar moiety (168), and another mutant, blocked in the pathway from tyrosine to ring B, indicated the sequence of assembly of the rings, C being added only after A and B are joined (O. K. Sebek, Abstr. 2nd Int. Cong. Genet. Ind. Microorg. 1974, p. 14). This mutant was also used in mutational biosynthesis to produce a novobiocin analog with a methyl group of the aminocoumarin replaced by chlorine.

Rifamycins. Rifamycins are the most important members of a class of antibiotics named ansamycins; they contain an aliphatic "ansa" chain bridging an aromatic chromophore. The rifamycins are a family of six or more compounds produced by *N. mediterranei*. Labeling experiments, more recently with ^{13}C-enriched precursors, have largely established the origin of the carbon skeleton of the rifamycins (172, 300). A 7-carbon aromatic ring would initiate synthesis of a polyketide chain derived from two malonates and eight methylmalonates; this polyketide would become the ansa chain and, together with the original aromatic ring, the chromophore. The product of this synthetic sequence should have a continuous carbon skeleton, yet the various rifamycins produced by the wild-type organism have an ether linkage interrupting the chain between carbons 12 and 29. A key finding was therefore the isolation, from a blocked mutant, of a novel compound, rifamycin W, lacking the ether linkage (301). This was shown to be converted to natural rifamycins and is, therefore, presumably a precursor of them, confirming the hypothesis that the ether linkage is introduced by interrupting the ansa chain after completion of the rifamycin carbon skeleton. Blocked mutants were also invaluable in elucidating a sequence from rifamycin W through SV (or S) to B (172).

A good system of genetic analysis is available in the rifamycin-producing strain of *N. mediterranei* (258, 259), but, unfortunately, no results of crosses involving blocked mutants have been published; thus, we are completely in the dark as to the genetic control of rifamycin biosynthesis.

Actinorhodin. This pigmented antibiotic is a symmetrical compound made of two identical

naphthoquinone half molecules (41). A priori it was likely to be synthesised by a polyketide route, and this has been made even more likely by establishment of such an origin for the nanaomycins of *Streptomyces rosa* var. *notoensis* (280), which closely resemble the half molecule of actinorhodin.

Actinorhodin was first studied in *S. coelicolor* strains of the type renamed *Streptomyces violaceoruber* (170), whereas strains of *S. coelicolor* (Müller) produce a completely different blue pigment, amylocyanin, and a different (heptaene) antibiotic (H. J. Kutzner et al., Abstr. 5th Int. Ferment. Symp. 1976, p. 235). Genetic interest in actinorhodin arises from the fact that it is produced in large quantities by *S. coelicolor* A3(2) (also a strain of the *S. violaceoruber* type; 308). A set of five blocked mutants was mapped to the chromosome of the strain presumptively in a gene cluster (308). These studies are being continued by B. A. M. Rudd, who has exploited the acid-base indicator properties of actinorhodin to isolate, by inspection of colonies, a large series of blocked mutants. These fall (B. A. M. Rudd, personal communication) into at least seven phenotypic classes on the basis of differences in precursor or shunt pigments and of cosynthesis tests, and all map to a region of the chromosome between the closely linked loci *hisD* and *guaA*. Recent isolation of an SCP1′ strain for this region (Rudd, personal communication) is allowing performance of dominance and complementation tests, while fine mapping of representative mutations should establish the sequence of genes in the cluster. This system, because of the well-developed experimental genetics of *S. coelicolor* A3(2), promises to be an excellent model for analysis of the genetic control of synthesis of a polyketide antibiotic. The strong evidence of gene clustering, taken with the less complete evidence for the tetracyclines, suggests that clustering may be a feature of genes controlling the biosynthesis of many antibiotics of this class.

Zorbamycin, etc. *Streptomyces bikiniensis* var. *zorbonensis* was found to produce several antibiotics, the structures of which appear not to have been published. Three mutations were mapped to a circular chromosomal linkage group (57): *zorD* lacked the ability to synthesize zorbamycin and zorbonomycins B and C, whereas *zorA* and *zorB* were also blocked in the production of some other antibiotics. In view of the scanty information available, few conclusions can be drawn from these results; conceivably *zorA* and *zorB* were pleiotropic mutations not directly involved in antibiotic synthesis [just as most bald mutations of *S. coelicolor* A3(2) fail to produce the chemically and genetically unrelated antibiotics methylenomycin and actinorhodin].

Plasmid involvement in antibiotic synthesis. *Methylenomycin in S. coelicolor.* The antimicrobial activity of this material was first described by Vivian (293); strains of *S. coelicolor* A3(2) known by genetic evidence to contain a sex factor (SCP1) inhibited the development of strains that had lost the plasmid. The inhibitor was later shown to be a low-molecular-weight, broad-spectrum material—a true antibiotic (164)—which was identified as methylenomycin A (307), a compound meanwhile described in another streptomycete (118). In *S. coelicolor* A3(2), the ability to produce methylenomycin A, and to be resistant to it, is completely correlated with the presence of SCP1, and this is also true of strains of *Streptomyces lividans* and *Streptomyces parvulus*, which lack SCP1 but to which it can be transferred by mating. More direct evidence that genes coding for biosynthetic enzymes are SCP1 linked comes from the isolation of presumed point mutations (*mmy*) that fail to produce the antibiotic. All 16 *mmy* mutations so far isolated were SCP1 linked (163). The number of genes represented is uncertain because a reliable complementation test for mutations on the low-copy-number SCP1 plasmid is not yet available, whereas cosynthetic reactions between the mutants were relatively few, with only one mutant acting as a converter. From cosynthesis data and some other phenotypic differences between the mutants, they were tentatively assigned to five groups; two were represented by single mutations, suggesting that further groups remain to be recognized.

Chemical studies on the biosynthetic pathway of methylenomycin A are so far lacking, and the *mmy* mutations are only now under biochemical investigation (U. Hornemann and D. A. Hopwood, unpublished data). However, an immediate precursor of methylenomycin A is apparently a compound postulated on mass spectral evidence to be the "des-epoxy" equivalent of the antibiotic (307) and is now identified as such (Hornemann and Hopwood, unpublished data). This compound is found, along with methylenomycin A, in SCP1-containing cultures of *S. coelicolor* A3(2), *S. lividans*, and *S. parvulus* (163, 307) and is converted to methylenomycin A by an *mmy* mutant of *S. coelicolor* A3(2) but not by SCP1$^-$ strains (Hornemann and Hopwood, unpublished data).

It will be very interesting to know the detailed genetic determination of methylenomy-

cin biosynthesis by *S. coelicolor* A3(2), in particular the number of biosynthetic steps involved and at what point the pathway diverges from primary metabolic reactions determined by chromosomal genes. This may be the first example, in any organism, of a comparatively extensive anabolic pathway controlled by plasmid-borne genes. It would also be extremely interesting to know if the corresponding genes are plasmid linked in other methylenomycin-producing streptomycetes; the strain described by Haneishi et al. (118) has apparently not been studied from the genetic point of view.

Chloramphenicol in S. venezuelae. Evidence for plasmid involvement in chloramphenicol biosynthesis was the failure of chloramphenicol-nonproducing variants (*cpp*), which arose with high frequency after acridine orange treatment, to map to a chromosomal linkage group that satisfactorily accommodated all auxotrophic mutations (3). The evidence is certainly suggestive, although the mapping procedure was unconventional because recombination frequencies were too low to distinguish recombinants differing from the parents by single markers from reverse mutations. No physical evidence for the putative plasmid has been published, and little information on its role in chloramphenicol biosynthesis is available. There is considerable biochemical evidence for a pathway of synthesis of chloramphenicol from shikimic acid (292). Mutants lacking an enzyme, arylamine synthetase, postulated to act early in the pathway, have been described (154) in a culture, *Streptomyces* sp. 3022a, presumed to be a strain of *S. venezuelae* (L. C. Vining, personal communication). A linkage map for this strain has been published (94), but no results of crosses involving *cpp* mutants are available. In the *S. venezuelae* strain, arylamine synthetase was lacking in one of the *cpp* mutants presumed to arise by plasmid loss (M. Okanishi, personal communication), but this result is, of course, equally compatible with the putative plasmid carrying regulatory rather than structural genes for chloramphenicol biosynthesis.

Turimycin in S. hygroscopicus. Evidence for plasmid involvement in the biosynthesis of this macrolide was the frequent origin of nonproducing variants, especially after acridine orange or ethidium bromide treatment (157). In crosses, the antibiotic production character was transferred to nonproducing variants at a much higher frequency than that of chromosomal recombination (D. Noack, personal communication). There is some physical evidence for the putative plasmid: antibiotic-producing cultures yielded different DNA profiles in gradient centrifugation compared with nonproducing variants (Noack, personal communication).

Kasugamycin and aureothricin in S. kasugaensis. A brief description of the frequent origin of strains that failed to produce one or other of these antibiotics after acridine orange or high-temperature treatment (225) provided the first suggestion that plasmids might be involved in the biosynthesis of streptomycete antibiotics, but, unfortunately, no genetic or physical evidence for the putative plasmid(s) has so far been published. Some DNA differences between the wild type and the nonproducing variants have been found (Okanishi, personal communication), and a full description of these results is awaited.

Oxytetracycline in S. rimosus. Although most classes of oxytetracycline-nonproducing (*otc*) mutations described by Boronin and coworkers mapped to a linkage group presumably representing the chromosome (see above), there was a class of nonproducers, also sensitive to the antibiotic, that arose with high frequency after acridine orange treatment (36) and showed evidence of nonlinkage with chromosomal markers (A. M. Boronin, A. N. Borisoglebskaya, and L. G. Sadovnikova, Abstr. 2nd Int. Symp. Genet. Ind. Microorg. 1974, p. 103). Further evidence that such strains arose by plasmid loss is needed, as is clarification of their biochemical lesion. It seems possible that all the structural genes for the biosynthetic pathway are chromosomal but that an extrachromosomal element is involved in the regulation of antibiotic biosynthesis or possibly export, a situation that may have a counterpart in the tyrosinase system of *Streptomyces glaucescens* (26). It is suggestive that a class of *otc* variant corresponding to the presumed extrachromosomal class was not found in another strain of *S. rimosus* by a second group of workers (J. Pigac, M. Vešligaj, and V. Delić, Abstr. 2nd Int. Symp. Genet. Ind. Microorg. 1974, p. 105).

A cautionary note. Not all phenotypes arising too frequently to be easily reconciled with gene mutation and failing to map to the chromosome need necessarily be due to plasmid loss. In *S. coelicolor* A3(2), chloramphenicol sensitivity (Cml^s) had such characteristics, but the powerful selection available for reversion to chloramphenicol resistance (Cml^r) allowed an oscillation between Cml^s and Cml^r to be demonstrated with no permanent loss of genetic information (95). Moreover, in contrast to the expectation for a self-transmissible plasmid, there was no infectious transfer of Cml^r into Cml^s

strains in crosses at frequencies greatly in excess of chromosomal marker transfer. This example, which is perhaps interpretable in terms of some kind of transposition of genetic information rather than its permanent loss, serves as a reminder that acceptable genetic and/or physical evidence is needed before a case of unstable antibiotic production is interpreted as necessarily due to plasmid loss.

Eubacteria

Peptide antibiotics of bacilli. Biochemical research has led to an understanding of the synthesis of several of these antibiotics, notably the cyclic decapeptides gramicidin S and tyrocidine of *B. brevis* (171, 180) and the cyclic dodecapeptide bacitracin A of *B. licheniformis* (99). Nonribosomal peptide bond formation is catalyzed by an antibiotic "synthetase" consisting of two or three subunits: "light" and "heavy" for gramicidin S (which may be considered a simpler molecule than the other two because it consists of two identical pentapeptides joined head to tail); "light," "intermediate," and "heavy" for tyrocidine; and A, B, and C for bacitracin. Peptide chain synthesis proceeds by sequential addition of amino acid residues to a growing chain, initiated at the N-terminal end. Sequence information is provided by the binding, through covalent linkage to enzyme -SH groups (thioester linkage), of each amino acid residue at a spatially distinct site on one or another of the enzyme subunits. Each subunit apparently contains a separate protein for each amino acid bound—one for the smallest subunits of gramicidin S and tyrocidine synthetases, which bind only phenylalanine, six for the largest component of tyrocidine synthetase, which binds six amino acids, etc. The growing peptide chain is handed on from one site to the amino acid bound at the next on the same or an adjacent subunit as appropriate. For certain phenylalanine residues of the two *B. brevis* antibiotics, racemization to the D-isomer also occurs at the binding site. Few details of cyclization of the completed peptides of tyrocidine and bacitracin and of the head-to-tail joining of the two pentapeptides of gramicidin S appear to be available. Nor is there information on the synthesis and attachment of the thiazoline ring present in bacitracin A.

These systems should provide fascinating objects for biochemical-genetic studies. The minimum number of gene products can be estimated. For example, for tyrocidine the three subunits contain one, three, and six proteins that bind amino acids, and in addition the two larger subunits have a small binding protein for phosphopantotheine, which plays a key role in peptide bond formation at each step in the synthesis (171, 180) (this is probably also true for bacitracin [247]), making about 12 gene products. Biochemical analysis of blocked mutants is proceeding, particularly for gramicidin S. Two Japanese groups have described series of such mutants. One group (158) classified five mutants into three functional classes by in vitro complementation using preparations of the two fractions of the synthetase. (Their fraction I is the "heavy" subunit referred to above, which activates four amino acids; fraction II is the "light" subunit, which activates and racemizes phenylalanine.) The three classes of mutants lacked the activity of one, the other, or both fractions. The other group (149) classified 20 mutants into three similar classes; later (269) these 20 mutants were tested for the ability of their fractions I and II to activate proline, valine, ornithine, and leucine (fraction I) or phenylalanine (fraction II), and five classes were found. In one, activation of all the amino acids occurred, whereas in another none was activated. A third class failed to activate phenylalanine and presumably had a lesion in fraction II. The other two classes were defective in fraction I function, failing to activate proline, valine, ornithine, and leucine or alternatively failing to activate only one of them (proline, valine, or leucine); this latter group presumably represented at least three classes of lesion in three different proteins of the fraction I subunit. The possibility of analyzing mutants of the cyclic peptide antibiotics functionally by these in vitro techniques makes the system very attractive, and it is disappointing that no system of genetic analysis is available in any of the producing strains.

Butirosin. This is a mixture of two aminoglycosides produced by some strains of *Bacillus circulans*. The technique of mutational biosynthesis (see above) was used with mutants blocked in the synthesis of the deoxystreptamine moiety (55, 282) by production of unnatural antibiotics containing streptamine, streptidine, or dideoxystreptamine. No genetic studies on the mutants have been reported. They would be of interest; could it be that the unique production of aminoglycosides by certain strains only of this eubacterial species indicates plasmid involvement?

Nisin. This linear polypeptide of 34 amino acids (116) is produced by *Streptococcus lactis* strains. Circumstantial evidence for plasmid involvement was the finding that certain strains gave up to 1% nisin-nonproducing cultures spontaneously and at very high frequency

after acridine orange, ethidium bromide, or high-temperature treatment (179). Certain of the nisin-negative derivatives lacked plasmid DNA present in their parent strains, but others did not (100). Thus, it is very likely that autonomous plasmids are involved in nisin production at least in some strains of *S. lactis*, and the results of further studies are eagerly awaited.

Prodigiosin. This pigment made by *Serratia marcescens* is probably the only antibiotic produced by a member of the *Enterobacteriaceae* that has been studied extensively (304). Large numbers of blocked mutants were isolated, and their study provided evidence for several features of the postulated biosynthetic pathway including its synthesis from two heterocyclic moieties, since reciprocal cosynthesis was observed between pairs of mutants, one blocked in the synthesis of each presumptive moiety. No recombination studies have, however, been made.

QUANTITATIVE GENETICS OF ANTIBIOTIC PRODUCTION

General Principles of Polygenic Control of Quantitative Characters

Many characters in all organisms show continuous variation; individuals from natural populations, progeny from a cross, or survivors of mutagenesis often form a continuous range of types inseparable into discrete classes. Such continuous variation is considered to result from the joint action of a number of "polygenes," each having a small effect in relation to the total genotypic variation. When these genetic effects are combined with the blurring effect of environmental influences, differences due to individual genes cannot be discerned by mere inspection of the population; but polygenes can be seen to segregate and obey the same Mendelian laws as the more readily identifiable "major" genes when analyzed by the techniques of "biometrical genetics." This is not an alternative to Mendelian genetics but a development of it that aims to determine the kinds of gene action and interaction involved in quantitative variation and to predict the outcome of future generations and the consequences of selection (85, 203, 204).

Biometrical genetics differs from Mendelian genetics in its analytical methods. Each generation comprises a continuous range of phenotypes, and, therefore, the properties of generations and the relationships between them are described statistically by means and variances as well as by correlations and covariances, respectively. By comparing these statistics with model expectations, the observed means and correlations (first-degree statistics) and variances and covariances (second-degree statistics) can be interpreted in terms of and partitioned into effects due to additive gene action, dominance, epistasis, linkage, environment, and interactions between genotype and environment.

Application of Biometrical Methods to Haploid and Nonmeiotic Systems

Microbial genetics have largely dealt with major gene differences where the inheritance of characters can be unambiguously analyzed qualitatively. Complications due to quantitative variation have been minimized by working mainly with closely related strains. The microbial geneticist investigating antibiotic production can usually choose neither the organism not the character for study and often cannot afford to ignore variation that is not discrete and that occurs in unrelated strains. Furthermore, antibiotic titer is often very sensitive to environmental variables so that even if the underlying genetic determination is relatively simple, the inherent discontinuities may be obscured by environmentally induced variation. Therefore, particularly when concerned with strain improvement for antibiotic production, biometrical genetics has a part to play along with the more widely recognized techniques of mutagenesis, recombination, and biochemical genetics.

Very little biometrical genetics has been done with microorganisms despite their many technical advantages. This may be due to the historical development of biometrical genetics in relation to plant and animal breeding and, hence, to diploid meiotic systems. An early attempt to analyze the polygenic nature of multistep chloramphenicol resistance in *E. coli* (54) has not been followed up, although genetic analysis in this organism is now much more refined. Recently, however, microorganisms have been used in the assessment of certain biometrical techniques (97, 98, 153), and the possibility of using large populations and of strictly defining environmental variables are important advantages.

The use of biometrical techniques to analyze quantitative variation in microorganisms was considered in detail by Caten and Jinks (53). The classical biometrical models for generation means and variances require complete diploid formation, unbiased segregation, and recovery of all possible genotypes in proportions determined by their linkage relationships; these requirements are clearly not satisfied in many microbial systems. However, classical biometrical methods can easily be applied to those eu-

caryotic microorganisms with a meiotic system, and the predominantly haploid life cycle simplifies analysis considerably since the complications of dominance do not occur. The application of biometrical techniques to a meiotic haploid system is exemplified below: the inheritance of penicillin production in *Aspergillus nidulans*.

Nonmeiotic systems. In all nonmeiotic systems, selection must be used to separate recombinant progeny from parentals. Normal biometrical methods do not apply because estimates such as those of the genetic component of variation are biased because all progeny carry a selected portion of the genome and are not a random sample of all possible genotypes. Comparisons of sexual and parasexual progeny in *A. nidulans* (53) showed considerable segregation and reassociation of polygenes during the parasexual cycle, but although differences between the two types of progeny sample were observed these may well have been due to selective bias in the parasexual analysis. Such bias could possibly be overcome by using more than one type of selection and reconstituting a random sample of genotypes from the resultant progeny.

Although nonmeiotic systems are not amenable to typical biometrical analysis, they could lend themselves to the study of quantitative variation by "chromosome assay" techniques. This involves the substitution of part of the genome, e.g., a chromosome or chromosome segment, from a selected strain into a tester strain while the rest of the genome is either held constant or allowed to vary randomly. If such substitution has a significant effect, then a gene or genes affecting the character are located in the tested region (202). A linkage map and some suitable selective markers are essential, and interpretation of the results may be complicated if the markers have pleiotropic effects on the character as is often found for antibiotic titer (89, 208). Nevertheless, many aspects of nonmeiotic systems are advantageous for this type of analysis. For example, the rarity of mitotic crossing-over in the parasexual cycle (20, 178, 237) means that whole chromosomes could be assayed by haploidization analysis without any significant intrachromosomal recombination. Such analysis could be particularly useful in strains in which antibiotic titer had been increased by mutagenesis, but its use in the study of natural quantitative variation in *Aspergillus* species has, unfortunately, been prevented by the heterokaryon incompatibility of unrelated isolates (52).

There are plenty of examples from strain improvement programs with antibiotic-producing actinomycetes of the stepwise increase in antibiotic titer over many rounds of mutation and selection (8). This in itself confirms the polygenic control of antibiotic titer. There are also some data from Alikhanian's group on the results of crosses between *S. rimosus* strains that indicate considerable variation in titer among the progeny of crosses even between closely related strains and more when wider crosses were made (8). However, the data were not analyzed by the techniques of biometrical genetics; nor was the segregation of individual genes affecting titer recognized (except the pleiotropic effects of the markers). These studies will therefore not be considered further.

Biometrical Genetics of Penicillin Production in *A. Nidulans*

Wild isolates of *A. nidulans* have been used in several studies of natural quantitative variation (46, 47, 152), and examples have been taken from studies of quantitative variation in penicillin production to demonstrate the type of information obtainable by biometrical analysis (for a more detailed account see references 208–210, 212). Holt and Macdonald (128) demonstrated that in three crosses between *A. nidulans* strains (one between two high-titer wild types and two between a wild type and a high-titer mutant) recombinants with substantially increased penicillin titers could be obtained. The distribution of titers among the recombinants indicated that penicillin production was probably under polygenic control, and this prompted a more detailed study of the amount of variation for penicillin titer present in natural populations. A random sample of wild isolates had a range of titers from 0.0 to 14.4 U/ml with a mean of 8.0 ± 0.4 U/ml (212). With suitable experimental designs and methods of analysis, the total phenotypic variation in an experiment may be partitioned into environmental (σ_E^2) and genetic (σ_G^2) components (53). About 60% of the observed variation among wild isolates was attributable to genetic differences between the isolates (212), indicating that there is a significant amount of heritable variation for penicillin titer in natural populations of *A. nidulans*.

In *A. nidulans*, heterokaryon formation between pairs of isolates is restricted to members of each heterokaryon compatibility (h-c) group (115), but sexual outcrossing is possible between all wild isolates to a greater or lesser extent regardless of h-c status (45). Thirty-one of the isolates tested had been allocated to h-c groups, and 67% of the variation in yield between isolates was due to differences between h-c groups (212).

To confirm genetic control of titer differences between wild isolates, crosses were made between representative isolates, and the titers of a sample of single ascospore cultures from each cross were determined. Such a cross can be used to investigate a number of factors, discussed in detail in reference 53. Estimates of heritability values (which indicate the proportion of the total observed variation fixable by selection [53]) varied between 0.0 and 0.77 in seven crosses between wild isolates (212). Of particular interest was the observation that crosses between isolates from different h-c groups had a much higher mean value for σ_G^2 (7.72) than crosses between isolates from the same h-c group (3.46). This reinforced the conclusions (47, 152) that isolates from the same h-c groups have similar genotypes and aided the choice of parental isolates for crosses to generate maximal genetic variation among the progeny.

The data can also be used to determine whether the segregating penicillin genes act additively or nonadditively. In a diploid organism, nonadditive variation can be due to either allelic interaction (dominance) or nonallelic interaction (epistasis), but in haploids all nonadditive variation must be due to epistasis. In *A. nidulans*, the gene action was predominantly additive, giving a symmetrical distribution of the progeny titers about a value close to the parental mean (212).

Knowledge of the type of gene action is particularly important when choosing a crossing scheme to select for increased titer. With mainly additive effects, it is simply necessary to combine the maximum number of "increasing" alleles into a single genotype, which can be readily achieved by a program of line selection (208, 209). If, instead, much of the gene action is epistatic, opportunity for free recombination should be provided from the outset of the program by maintaining a single breeding population on which selection is practiced.

The influence of genetic markers on antibiotic production can also be examined biometrically. In crosses with *A. nidulans*, progeny with yellow conidia were often found to have a lower mean titer than those with green, and it was shown that pleiotropy of the *y* mutation rather than linkage of this allele to genes for decreased titer was the causative factor (208). On the other hand, linkage of polygenes to known chromosomal markers has been demonstrated by biometrical analysis in *Schizophyllum commune* (58), *Neurospora crassa* (230), and *Aspergillus amstelodami* (53).

Having shown that significant genetic variation can be released by crosses, one can attempt to recombine such variation to generate strains with improved titers. In *A. nidulans*, four selection lines were initiated, each from a cross between two wild isolates. Each parental strain was chosen for high titer and for heterokaryon incompatibility with the other seven strains so as to maximize the available genetic variation in the initial gene pool. During selection for three to five generations, the mean progeny titer approximately doubled in each line. The available genetic variation was significantly reduced in all four lines by generation 4 or 5 (208). Such a response is expected in a haploid organism when, as in this case, a scheme of sibling mating is used. But the expectation was that if different genes for increased titer had been accumulated in the different selection lines, further genetic variation should have been releasable by crossing selected strains from different lines.

This was confirmed by crosses between representatives from three of the *A. nidulans* selection lines, and in each case a substantial amount of genetic variation, equal to that in the original crosses between wild isolates, was released (209). Moreover, the gene action in interline crosses remained additive so that new high-titer genotypes were generated. It was then possible to use further rounds of crossing and selection to obtain further increases in titer. By introducing new sources of genetic variation when necessary, such stepwise increases in titer might be continued over several cycles.

The biometrical methods applied here to a system of crossing and selection based on natural variation are equally applicable to one based on mutationally induced variation, and such an analysis has recently been carried out (I. Simpson and C. E. Caten, personal communication). Mutation and selection were used to increase penicillin production by *A. nidulans* in two independent selection lines, one treated with 8-methoxypsoralen plus near-UV and the other with ethylmethanesulfonate. Six cycles of selection resulted in increases in titer from 5 to 15 U/ml and from 7 to 18 U/ml. The induced titer-increasing mutations either were recessive or showed ambidirectional dominance in diploids. Biometrical analysis suggested that the increased titers were probably due to mutations in several genes and that the mutations in the two selection lines were not allelic. Crosses between the selection lines showed slight epistasis, with a progeny mean slightly lower than the midparental value, but further cycles of selection and hybridization initiated from this cross produced recombinant strains with improved titers over 20 U/ml.

Hence, in this system there appear to be a no inherent differences between naturally occurring and mutagenically induced variation, and both can be successfully analyzed by biometrical techniques. Even in schemes where hybridization is not employed, the use of biometrical methods to monitor available genetic variation would be of considerable value both in comparing the efficiency of different mutagenic treatments and in assessing the amount of variation available for selection.

Quantitative Mutations Affecting Penicillin Yield

All commerical strains of *P. chrysogenum* derive from the Wisconsin family, the result of a vast mutation program at the Botany Department of Wisconsin University between 1946 and 1956, which gave at least a fivefold improvement in penicillin titer (16). Further large yield increments occurred later in commercial strain development programs (83). The genetic changes that led to these increments have been studied extensively.

Heterokaryons between high- and low-titer strains produced segregants in which penicillin titer was associated with parental chromosomal markers (190, 262), indicating the yield mutations to be nuclear rather than cytoplasmic. Diploids between high-titer strains and their progenitors invariably had titers similar to those of the low-yielding strain (261). Thus positive mutations, including such significant events as that differentiating strains WisQ176 and X1612, were all recessive. Diploids between a wild-type strain (NRRL 1951) and two derivatives selected after a number of cycles of mutagenesis with several mutagens to produce about 3,000 U more than the wild type all had titers similar to or only slightly greater than the wild type. Hence, again, the positive mutations were almost completely recessive (190).

Diploids between strains carrying independent positive mutations usually had yields equal or inferior to that of the less productive parent (192, 263, 264). If all the mutations had been recessive and nonallelic, the diploids should have had a yield similar to that of the common ancestor; thus, many of the mutations were allelic (192). However, one diploid between strains Wis49-133 and Wis50-1247 had a consistently higher yield than the component strains (262). This result is, of course, difficult to explain unless dominant mutations for increased yield were involved, and a satisfactory explanation has not been put forward.

Large variations in titer occurred among diploids between the same two high-titer parents (184, 192). The yields of 68 diploids, all synthesized from the same two parents, yielding 3,000 and 3,500 U, fell into two distinct groups; 44 yielded around 600 U/ml and 24 about 3,000 U/ml. Two explanations were proposed. In one (184), the high-titer diploids were normal and those of low titer arose by dominant mutations reducing yield and conferring a selective advantage on the resultant low-titer strains. This hypothesis predicted that variability should also arise in diploids homozygous for penicillin mutations, but a study of such diploids (184) was inconclusive. In an alternative hypothesis (264), the high-titer diploids arose from those of low titer by segregation, which would have had to be transitory and had to occur soon after diploidization since isolated low-yielding diploids did not spontaneously segregate high-titer diploids. Low-titer diploids did produce high-titer, first-order haploid segregants (184). This interpretation requires some lack of allelism between positive mutations. Conclusive proof of either hypothesis would be difficult to obtain.

Any unusual segregation data may reflect that many if not all improved strains may carry chromosome aberrations arising from the mutagenic treatments used in selection programs. Such aberrations have not been conclusively demonstrated, but several pieces of evidence indicate that they are common in improved strains. Genetic analysis in *A. nidulans* demonstrated that chromosome aberrations can be induced at a significant frequency by UV and X-irradiation (17, 156), and the spontaneous production of a variety of morphological types (termed "population patterns" [276]) by many improved strains of *P. chrysogenum* is reminiscent of the morphological effects of chromosome translocations or duplications in *A. nidulans* (156, 288). Diploids between pairs of high-yielding strains invariably produced a majority of haploid segregants of one or other parental phenotype (192, 193). This phenomenon, termed "parental genome segregation," (192) is probably due to differences in chromosome structure between the parent strains (185).

Haploid segregants from a heterozygous diploid never had a significantly higher titer than both parent strains (192). However, haploids with titers significantly lower than both parents often arose presumptively by recombination of deleterious recessive mutations from both parents (263).

The studies described so far dealt with strains carrying one or more unidentified mutations causing increased penicillin yield. More recently, experiments have been carried out with *P. chrysogenum* and *A. nidulans* in which

single titer-increasing mutations were mapped, and the effects of recombining such mutations into a single strain were studied.

P. chrysogenum. Some of the earlier problems of parasexual analysis in *P. chrysogenum*—parental genome segregation and possible selection against certain segregants (185, 192, 193, 262)—were overcome by using sister strains differing in only a few mutational steps (20). After haploidization by PFA treatment, 21 independently isolated spore color and auxotrophic markers were allocated to one or other of three "haploidization groups." Strains carrying these markers were then used as parents in which single-step titer increases were induced by UV. The parents had titers of ca. 3,000 U/ml, and the mutations caused increases of 500 to 2,000 U/ml. Attempts were made to allocate these mutations to haploidization groups by crossing with other marked strains. Many segregants had titers considerably lower than the parents. Free recombination was observed between haploidization groups, but this recombination apparently disrupted the balanced polygenic systems responsible for the high titers in the parent strains. Unfortunately these effects, combined with the very low numbers of segregants scored (sometimes less than 10), made the unambiguous allocation of titer-increasing mutations to haploidization groups rather difficult.

On the basis of tentative allocations, strains carrying different mutations were crossed in an attempt to obtain segregants carrying both mutations (21, 22); two crosses produced such segregants with significantly increased titers. The effects of the two mutations were completely additive, a promising result for the future application of recombination to titer improvement. Segregants with much lower titers also arose, but the authors did not comment on the stability of the high-titer recombinants.

A. nidulans. Three mutations causing increased penicillin titer in *A. nidulans*, *penA1*, *penB2*, and *penC3*, had titers of 20, 12, and 20 U/ml, respectively, compared with 6 U/ml for the parental strain (NRRL 194) (127, 188). Each mutation was mapped to its chromosome by parasexual. haploidization analysis (207): *penA1*, *penB2*, and *penC3* to chromosomes VIII, III, and IV, respectively. They were mapped relative to other loci on the chromosome by cleistothecial analysis (76). *PenA1* was tentatively placed equidistant between *chaA* and *nirA* at the distal end of the right arm of chromosome VIII. Strains carrying *penB2* were morphologically abnormal, and genetic analysis indicated that this might have been a pleiotropic effect of *penB2*, which was located 13 map units from *moC* on the left arm of chromosome III. Strains carrying *penC3* were apparently infertile, but hybrid perithecia were obtained by crossing with a strain carrying the mutation *sgpC*. Crosses between strains bearing allelic *sgp* mutations are sexually sterile, but those between nonallelic *sgp* mutations are fertile (148), and *penC3* appears, therefore, to mimic the behavior of *sgp* mutations. By crossing a strain carrying *penC3* with one carrying an *sgp* mutation, fertility was achieved, and *penC3* was shown to be linked to *pyroA* on the right arm of chromosome IV.

The titer of heterozygous diploids between each of the three mutations and a wild-type strain suggested that *penA1* was recessive, *penB2* dominant, and *penC3* semidominant (76). Recessivity of *penA1* was confirmed in a *penA1 penB2* (*trans*) heterozygous diploid, which had a titer similar to that of the *penB2* parent. A *penB2 penC3* (*trans*) heterozygous diploid also had the same titer as the *penB2* parent, and no additive effect of the two mutations was found. The titers of haploid recombinants carrying two of the three mutations in all three combinations indicated that *penA1* was epistatic to *penB2* and *penC3* and that *penC3* was epistatic to *penB2*. The lack of additivity between titer-increasing mutations in this study contrasts with the marked additivity found in *P. chrysogenum* (22) and in studies of natural variation in titer in *A. nidulans* (see above).

CONCLUSION

The Application of Genetic Knowledge

This review has highlighted the discrepancy between the amount of knowledge of the genetic control of antibiotic production so far obtained and the potential for obtaining such knowledge. A similar gulf separates the potential for harnessing of genetics in the development of new industrial strains and the use that has been made of genetics in such endeavors. The paucity of knowledge accounts, in part, for the dearth of applications. However, especially with the opening of the era of genetic engineering, strain improvement will increasingly demand answers to questions about the number, arrangement, and roles of genes determining antibiotic production if the new techniques are to be used effectively (138). We can expect, therefore, a big increase in research in this area in the near future.

ACKNOWLEDGMENTS

We are grateful to all those, mentioned in the text, who kindly supplied us with their unpublished data. We should particularly like to acknowledge

Georgina Moore for expert secretarial assistance in the preparation of this article.

LITERATURE CITED

1. **Achenbach, H.** 1967. Xanthocillin, p. 26-28. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics: biosynthesis, vol. 2. Springer-Verlag, Berlin, Heidelberg, and New York.
2. **Adams, J. N., and G. H. Brownell.** 1976. Genetic studies in *Nocardia erythropolis*, p. 285-309. *In* M. Goodfellow, G. H. Brownell, and J. A. Serrano (ed.), The biology of the nocardiae. Academic Press, London, New York, and San Francisco.
3. **Akagawa, H., M. Okanishi, and H. Umezawa.** 1975. A plasmid involved in chloramphenicol production in *Streptomyces venezuelae*: evidence from genetic mapping. J. Gen. Microbiol. **90**:336-346.
4. **Alačević, M.** 1963. Interspecific recombination in *Streptomyces*. Nature (London) **197**:1323.
5. **Alačević, M.** 1976. Recent advances in *Streptomyces rimosus* genetics, p. 513-519. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
6. **Alačević, M., M. Strašek-Vešligaj, and G. Sermonti.** 1973. The circular linkage map of *Streptomyces rimosus*. J. Gen. Microbiol. **77**:173-185.
7. **Alačević, M., D. Vlasić, and I. Spada-Sermonti.** 1966. Interspecific recombination among antibiotic-producing streptomycetes, p. 720-722. *In* M. Herold and D. Zdenek (ed.), Antibiotics: advances in research, production and clinical use. Butterworths, London.
8. **Alikhanian, S.** 1970. Applied aspects of microbial genetics. Curr. Top. Microbiol. and Immunol. **53**:91-148.
9. **Alikhanian, S. I., and L. N. Borisova.** 1961. Recombination in *Actinomyces aureofaciens*. J. Gen. Microbiol. **26**:19-28.
10. **Alikhanian, S. I., T. S. Ilyina, and N. D. Lomovskaya.** 1960. Transduction in actinomycetes. Nature (London) **188**:245-246.
11. **Alikhanian, S. I., N. D. Lomovskaya, and V. N. Danilenko.** 1976. Suppressor-sensitive mutations of *Streptomyces coelicolor* A3(2) and actinophage ϕC31, p. 595-606. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
12. **Anné, J., H. Eyssen, and P. De Somer.** 1976. Somatic hybridisation of *Penicillium roquefortii* with *P. chrysogenum* after protoplast fusion. Nature (London) **262**: 719-721.
13. **Anné, J., and J. F. Peberdy.** 1976. Induced fusion of fungal protoplasts following treatment with polyethylene glycol. J. Gen. Microbiol. **92**:413-417.
14. **Arnstein, H. R. V., D. Morris, and E. J. Toms.** 1959. Isolation of a tripeptide containing α-aminoadipic acid from the mycelium of *Penicillium chrysogenum* and its possible significance in penicillin biosynthesis. Biochim. Biophys. Acta **35**:561-562.
15. **Audit, C., and C. Anagnostopoulos.** 1973. Genetic studies relating to the production of transformed clones diploid in the tryptophan region of the *Bacillus subtilis* genome. J. Bacteriol. **114**:18-27.
16. **Backus, M. P., and J. F. Stauffer.** 1955. The production and selection of a family of strains in *Penicillium chrysogenum*. Mycologia **47**:429-463.
17. **Bainbridge, B. W., and J. A. Roper.** 1966. Observations on the effects of chromosome duplication in *Aspergillus nidulans*. J. Gen. Microbiol. **42**:417-424.
18. **Bal, J., E. Bartnik, B. Goryluk, and N. J. Pieniazek.** 1975. An easy way of obtaining *Aspergillus nidulans* haploids in the parasexual cycle using *N*-glycosyl polifungin. Genet. Res. **25**:249-252.
19. **Balassa, G.** 1969. Biochemical genetics of bacterial sporulation. I. Unidirectional pleiotropic interactions among genes controlling sporulation in *Bacillus subtilis*. Mol. Gen. Genet. **104**:73-103.
20. **Ball, C.** 1971. Haploidisation analysis in *Penicillium chrysogenum*. J. Gen. Microbiol. **66**:63-69.
21. **Ball, C.** 1973. Improvement of penicillin productivity in *Penicillium chrysogenum* by recombination, p. 227-237. *In* Z. Vaněk, Z. Hošťálek, and J. Cudlín (ed.), Genetics of industrial microorganisms: actinomycetes and fungi. Academia, Prague.
22. **Ball, C., and J. L. Azevedo.** 1976. Genetic instability in parasexual fungi, p. 243-251. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
23. **Bardone, M. R., E. Martinelli, L. F. Zerilli, and C. Coronelli.** 1974. Structure determination of purpuromycin, a new antibiotic. Tetrahedron **30**:2747-2754.
24. **Batchelor, F. R., F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson.** 1959. Synthesis of penicillin: 6-aminopenicillanic acid in penicillin fermentation. Nature (London) **183**:257-258.
25. **Baumann, R., R. Hütter, and D. A. Hopwood.** 1974. Genetic analysis in a melanin-producing streptomycete, *Streptomyces glaucescens*. J. Gen. Microbiol. **81**:463-474.
26. **Baumann, R., and H. P. Kocher.** 1976. Genetics of *Streptomyces glaucescens* and regulation of melanin production, p. 535-552. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
27. **Bérdy, J.** 1974. Recent developments of antibiotic research and classification of antibiotics according to chemical structure. Adv. Appl. Microbiol. **18**:309-406.
28. **Beretta, M., M. Betti, and M. Polsinelli.** 1971. Genetic recombination in micromonospora.

J. Bacteriol. 107:415-419.
29. **Bibb, M. J., R. F. Freeman, and D. A. Hopwood.** 1977. Physical and genetical characterisation of a second sex factor, SCP2, for *Streptomyces coelicolor*. Mol. Gen. Genet. 154:155-166.
30. **Birch, A. J.** 1967. Biosynthesis of polyketides and related compounds. Science 156:202-206.
31. **Birch, A. J.** 1967. Fumagillin, p. 152-153. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics: biosynthesis, vol. 2. Springer-Verlag, Berlin, Heidelberg, and New York.
32. **Blumauerová, M., Ž. Hošťálek, and Z. Vaněk.** 1972. Biosynthesis of tetracyclines: problems and perspectives in genetic analysis, p. 223-232. *In* G. Terni (ed.), Fermentation technology today. Osaka Society of Fermentation Technology, Osaka.
33. **Bonner, D.** 1947. Studies on the biosynthesis of penicillin. Arch. Biochem. 13:1-9.
34. **Bordet, C., M. Karahjoli, O. Gateau, and G. Michel.** 1972. Cell walls of nocardiae and related actinomycetes: identification of the genus *Nocardia* by cell wall analysis. Int. J. Syst. Bacteriol. 22:251-259.
35. **Boronin, A. M., and S. Z. Mindlin.** 1971. Genetic analysis of *Actinomyces rimosus* mutants with impaired synthesis of antibiotic (in Russian). Genetika 7:125-131.
36. **Boronin, A. M., and L. G. Sadovnikova.** 1972. Elimination by acridine dyes of oxytetracycline resistance in *Actinomyces rimosus* (in Russian) Genetika 8:174-176.
37. **Bousfield, I. J., and M. Goodfellow.** 1976. The "Rhodochrous" complex and its relationships with allied taxa, p. 39-65. *In* M. Goodfellow, G. H. Brownell, and J. A. Serrano (ed.), The biology of the nocardiae. Academic Press, London, New York, and San Francisco.
38. **Bradley, D. E.** 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. 31:230-314.
39. **Bradley, S. G., and J. Lederberg.** 1956. Heterokaryosis in *Streptomyces*. J. Bacteriol. 72:219-225.
40. **Braendle, D. H., and W. Szybalski.** 1959. Heterokaryotic compatibility, metabolic cooperation, and genic recombination in *Streptomyces*. Ann. N.Y. Acad. Sci. 81:824-851.
41. **Brockmann, H., A. Zeeck, K. van der Merwe, and W. Müller.** 1966. Über Actinomycetenfarbstoffe, VIII. Die Konstitution des Antinorhodins. Liebigs Ann. Chem. 698:209-229.
42. **Bu'lock, J. D.** 1961. Intermediary metabolism and antibiotic synthesis. Adv. Appl. Microbiol. 3:293-342.
43. **Bu'lock, J. D.** 1975. Secondary metabolism in fungi and its relationships to growth and development, p. 33-58. *In* J. E. Smith, and D. R. Berry (ed.), The filamentous fungi: industrial mycology, vol. 1. Edward Arnold, London.
44. **Bu'lock, J. D., D. Hamilton, M. A. Hulme, A. J. Powell, H. M. Smalley, D. Shepherd, and G. N. Smith.** 1965. Metabolic development and secondary biosynthesis in *Penicillium urticae*. Can. J. Microbiol. 11:765-778.
45. **Butcher, A. C.** 1968. The relationship between sexual outcrossing and heterokaryon incompatibility in *Aspergillus nidulans*. Heredity 23:443-452.
46. **Butcher, A. C.** 1969. Non-allelic interactions and genetic isolation in wild populations of *Aspergillus nidulans*. Heredity 24:621-631.
47. **Butcher, A. C., J. Croft, and M. Grindle.** 1972. Use of genotype-environmental interaction analysis in the study of natural populations of *Aspergillus nidulans*. Heredity 29:263-283.
48. **Caglioti, M. T., and G. Sermonti.** 1956. A study of the genetics of penicillin-producing capacity in *Penicillium chrysogenum*. J. Gen. Microbiol. 14:38-46.
49. **Calam, C. T., L. B. Daglish, and W. S. Gaitskell.** 1973. Hybridisation experiments with *Penicillium patulum* and *Fusarium moniliforme*, p. 265-282. *In* Z. Vaněk, Z. Hošťálek, and J. Cudlín (ed.), Genetics of industrial microorganisms: actinomycetes and fungi. Academia, Prague.
50. **Caltrider, P. G., and H. F. Niss.** 1966. Role of methionine in cephalosporin synthesis. Appl. Microbiol. 14:746-753.
51. **Carere, A., and R. Randazzo.** 1976. Co-mutation in *Streptomyces*, p. 573-581. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
52. **Caten, C. E., and J. L. Jinks.** 1966. Heterokaryosis: its significance in wild homothallic ascomycetes and fungi imperfecti. Trans. Br. Mycol. Soc. 49:81-93.
53. **Caten, C. E., and J. L. Jinks.** 1976. Quantitative genetics, p. 93-111. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
54. **Cavalli, L. L., and G. A. Maccacaro.** 1952. Polygenic inheritance of drug-resistance in the bacterium *Escherichia coli*. Heredity 6:311-331.
55. **Claridge, C. A., J. A. Bush, M. D. Defuria, and K. E. Price.** 1974. Fermentation and mutation studies with a butirosin-producing strain of *Bacillus circulans*. Dev. Ind. Microbiol. 15:102-113.
56. **Coats, J. H.** 1976. Genetic recombination in *Streptomyces achromogenes* var. *rubradiris*, p. 521-530. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
57. **Coats, J. H., and J. Roeser.** 1971. Genetic recombination in *Streptomyces bikiniensis* var. *zorbonensis*. J. Bacteriol. 105:880-885.
58. **Connolly, V., and G. Simchen.** 1968. Linkage to the incompatibility factors and maintenance of genetic variation in selection lines

of *Schizophyllum commune*. Heredity 23:387-402.

59. **Corcoran, J. W.** 1974. Lipid and macrolide lactone biosynthesis in *Streptomyces erythreus*. Dev. Ind. Microbiol. 15:93-100.
60. **Coronelli, C., G. Tamoni, and G. C. Lancini.** 1976. Gardimycin, a new antibiotic from *Actinoplanes*. II. Isolation and preliminary characterisation. J. Antibiot. 29:507-510.
61. **Coronelli, C., R. J. White, G. C. Lancini, and F. Parenti.** 1975. Lipiarmycin, a new antibiotic from *Actinoplanes*. II. Isolation, chemical, biological and biochemical characterisation. J. Antibiot. 28:253-259.
62. **Cove, D. J.** 1976. The control of catabolism in *Aspergillus nidulans*, p. 407-418. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
63. **Cross, T., and R. W. Atwell.** 1975. Actinomycete spores, p. 3-14. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
64. **Cross, T., and M. Goodfellow.** 1973. Taxonomy and classification of the actinomycetes, p. 11-112. *In* G. Sykes, and F. A. Skinner (ed.), Actinomycetales: characteristics and practical importance. Academic Press, London.
65. **Crowdy, S. H., J. F. Grove, and P. McClosky.** 1959. The translocation of antibiotics in higher plants. IV. Systemic fungicidal activity and chemical structure in griseofulvin relatives. Biochem. J. 72:241-249.
66. **de Bertoldi, M., and C. E. Caten.** 1975. Isolation and haploidisation of heterozygous diploid strains in species of *Humicola*. J. Gen. Microbiol. 91:63-73.
67. **Delić, V.** 1965. Genetic recombination in *Streptomyces erythreus* (in Serbo-Croat). Mikrobiologija 2:153-156.
68. **Demain, A. L.** 1957. Inhibition of penicillin formation by lysine. Arch. Biochem. Biophys. 67:244-245.
69. **Demain, A. L.** 1966. Industrial fermentations and their relation to regulatory mechanisms. Adv. Appl. Microbiol. 8:1-27.
70. **Demain, A. L.** 1974. Biochemistry of penicillin and cephalosporin fermentations. Lloydia 37:147-167.
71. **Demain, A. L.** 1974. How do antibiotic producing microorganisms avoid suicide? Ann. N.Y. Acad. Sci. 235:601-602.
72. **Demain, A. L., and E. Inamine.** 1970. Biochemistry and regulation of streptomycin and mannosidostreptomycinase (α-D-mannosidase) formation. Bacteriol. Rev. 34:1-19.
73. **Demain, A. L., and P. S. Masurekar.** 1974. Lysine inhibition of *in vivo* homocitrate synthesis in *Penicillium chrysogenum*. J. Gen. Microbiol. 82:143-151.
74. **Dennen, D. W., and D. D. Carver.** 1969. Sulfatase regulation and antibiotic synthesis in *Cephalosporium acremonium*. Can. J. Microbiol. 15:175-181.
75. **Dimroth, P., E. Ringelmann, and F. Lynen.** 1976. 6-Methylsalicylic acid synthetase from *Penicillium patulum*. Eur. J. Biochem. 68:591-596.
76. **Ditchburn, P., G. Holt and K. D. Macdonald.** 1976. The genetic location of mutations increasing penicillin yield in *Aspergillus nidulans*, p. 213-227. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
77. **Dobrzanski, W. T., and H. Osowiecki.** 1967. Isolation and some properties of the competence factor from group H streptococcus strain CHALLIS. J. Gen. Microbiol. 48:299-304.
78. **Drew, S. W., and A. L. Demain.** 1973. Methionine control of cephalosporin C formation. Biotech. Bioeng. 15:743-754.
79. **Drew, S. W., and A. L. Demain.** 1975. Production of cephalosporin C by single and double sulfur auxotrophic mutants of *Cephalosporium acremonium*. Antimicrob. Agents Chemother. 8:5-10.
80. **Drew, S. W., and A. L. Demain.** 1975. The obligatory role of methionine in the conversion of sulfate to cephalosporin C. Eur. J. Appl. Microbiol. 1:121-128.
81. **Dulaney, E. L.** 1969. Variation in selected populations of tetracycline producers, p. 93-120. *In* G. Sermonti and M. Alačević (ed.), Genetics and breeding of streptomyces. Yugoslav Academy of Sciences and Arts, Zagreb.
82. **Dulaney, E. L., I. Putter, D. Drescher, L. Chaiet, W. J. Miller, F. J. Wolf, and D. Hendlin.** 1962. Transethylation in antibiotic biosynthesis. I. An ethyl homolog of oxytetracycline. Biochim. Biophys. Acta 60:447-449.
83. **Elander, R. P.** 1967. Enhanced penicillin biosynthesis in mutant and recombinant strains of *Penicillium chrysogenum*. Abh. Dtsch. Akad. Wiss. Berlin Kl. Med. 2:403-423.
84. **Elander, R. P., M. A. Espenshade, S. G. Pathak, and C. H. Pan.** 1973. The use of parasexual genetics in an industrial strain improvement program with *Penicillium chrysogenum*, p. 239-253. *In* Z. Vaněk, Z. Hoštálek, and J. Cudlín (ed.), Genetics of industrial microorganisms: actinomycetes and fungi. Academia, Prague.
85. **Falconer, D. S.** 1960. Quantitative genetics. Oliver and Boyd, Edinburgh and London.
86. **Falkow, S.** 1975. Infectious multiple drug resistance. Pion Ltd., London.
87. **Fantini, A. A.** 1962. Genetics and antibiotic production of *Emericellopsis* species. Genetics 47:161-177.
88. **Fantini, A. A., and L. S. Olive.** 1960. Sexual recombination in a homothallic, antibiotic producing fungus. Science 132:1670.
89. **Fawcett, P. A., J. J. Usher, and E. P. Abraham.** 1976. Aspects of cephalosporin and pen-

icillin biosynthesis, p. 129-138. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.

90. **Ferenczy, L., F. Kevei, M. Szegedi, A. Frankó, and I. Rojik.** 1976. Factors affecting high-frequency fungal protoplast fusion. Experientia 32:1156-1158.
91. **Fincham, J. R. S., and P. R. Day.** 1971. Fungal genetics, 3rd ed. Blackwell Scientific Publications, Oxford and Edinburgh.
92. **Flynn, E. H., M. H. McCormick, M. C. Stamper, H. de Valeria, and C. W. Godzeski.** 1962. A new natural penicillin from *Penicillium chryosgenum*. J. Am. Chem. Soc. 84:4594-4595.
93. **Forrester, P. I., and G. M. Gaucher.** 1972. Conversion of 6-methylsalicylic acid into patulin by *Penicillium urticae*. Biochemistry 11:1102-1107.
94. **Francis, M. M., R. Cella, and L. C. Vining.** 1975. Genetic recombination in a chloramphenicol-producing strain of *Streptomyces* species 3022A. Can. J. Microbiol. 21:1151-1159.
95. **Freeman, R. F., M. J. Bibb, and D. A. Hopwood.** 1977. Chloramphenicol acetyltransferase-independent chloramphenicol resistance in *Streptomyces coelicolor* A3(2). J. Gen. Microbiol. 98:453-465.
96. **Friend, E. J., and D. A. Hopwood.** 1971. The linkage map of *Streptomyces rimosus*. J. Gen. Microbiol. 68:187-197.
97. **Fripp, Y. J.** 1972. Genotype-environmental interactions in *Schizophyllum commune*. II. Assessing the environment. Heredity 28:223-238.
98. **Fripp, Y. J., and C. E. Caten.** 1971. Genotype-environmental interactions in *Schizophyllum commune*. I. Analysis and character. Heredity 27:393-407.
99. **Frøyshov, Ø.** 1975. Enzyme-bound intermediates in the biosynthesis of bacitracin. Eur. J. Biochem. 59:201-206.
100. **Fuchs, P. G., J. Zajdel, and W. T. Dobrzanski.** 1975. Possible plasmid nature of the determinant for production of the antibiotic nisin in some strains of *Streptomyces lactis*. J. Gen. Microbiol. 88:189-192.
101. **Fujisawa, Y., and T. Kanzaki.** 1975. Occurrence of a new cephalosporoate in a culture broth of a *Cephalosporium acremonium* mutant. J. Antibiot. 28:372-378.
102. **Fujisawa, Y., K. Kitano, and T. Kanzaki.** 1975. Accumulation of deacetoxycephalosporin C by a deacetylcephalosporin C negative mutant of *Cephalosporium acremonium*. Agric. Biol. Chem. 39:2049-2055.
103. **Fujisawa, Y., H. Shirafuji, and T. Kanzaki.** 1975. Deacetylcephalosporin C formation by cephalosporin C acetylhydrolase induced in a *Cephalosporium acremonium* mutant. Agric. Biol. Chem. 39:1303-1309.
104. **Fujisawa, Y., H. Shirafuji, M. Kida, K. Nara, M. Yoneda, and T. Kanzaki.** 1973. New findings on cephalosporin C biosynthesis. Nature (London) New Biol. 246:154-155.
105. **Fujisawa, Y., H. Shirafuji, M. Kida, K. Nara, M. Yoneda, and T. Kanzaki.** 1975. Accumulation of deacetylcephalosporin C by cephalosporin C negative mutants of *Cephalosporium acremonium*. Agric. Biol. Chem. 39:1295-1301.
106. **Furumai, T., and M. Suzuki.** 1975. Studies on the biosynthesis of basic 16-membered macrolide antibiotics, platenomycins. I. Selection of and cosynthesis by non-platenomycin-producing mutants. J. Antibiot. 28:770-774.
107. **Furumai, T., K. Takeda, and M. Suzuki.** 1975. Studies on the biosynthesis of basic 16-membered macrolide antibiotics, platenomycins. IV. Biosynthesis of platenomycins. J. Antibiot. 28:789-797.
108. **Furuya, K., R. Enokita, and M. Shirasaka.** 1967. Antibiotics from fungi. II. A new griseofulvin producer, *Nigrospora oryzae*. Annu. Rep. Sankyo Res. Lab. 19:91-95.
109. **Godtfredsen, W. O., S. Jahnsen, H. Lorck, K. Roholt, and L. Tybring.** 1962. Fusidic acid: a new antibiotic. Nature (London) 193:987.
110. **Gordon, R. E., D. A. Barnett, J. E. Handerhan, and C. H. Pang.** 1974. *Nocardia coeliaca, Nocardia autotrophica* and the nocardin strain. Int. J. Syst. Bacteriol. 24:54-63.
111. **Goulden, S. A., and F. W. Chattaway.** 1968. Lysine control of α-aminoadipate and penicillin synthesis in *Penicillium chrysogenum*. Biochem. J. 110:55P.
112. **Goulden, S. A., and F. W. Chattaway.** 1969. End-product control of acetohydroxyacid synthetase by valine in *Penicillium chrysogenum* Q176 and a high penicillin-yielding mutant. J. Gen. Microbiol. 59:111-118.
113. **Gregory, K. F., and J. C. C. Huang.** 1964. Tyrosinase inheritance in *Streptomyces scabies*. I. Genetic recombination. J. Bacteriol. 87:1281-1286.
114. **Gregory, K. F., and J. C. C. Huang.** 1964. Tyrosinase inheritance in *Streptomyces scabies*. II. Induction of tyrosinase deficiency by acridine dyes. J. Bacteriol. 87:1287-1294.
115. **Grindle, M.** 1963. Heterokaryon compatibility of unrelated strains in the *Aspergillus nidulans* group. Heredity 18:191-204.
116. **Gross, E., and J. L. Morell.** 1971. The structure of nisin. J. Am. Chem. Soc. 93:4634-4635.
117. **Haavik, H., and Ø. Frøyshov.** 1975. Function of peptide antibiotics in producer organisms. Nature (London) 254:79-81.
118. **Haneishi, T., A. Terahara, M. Arai, T. Hata, and C. Tamura.** 1974. New antibiotics, methylenomycins A & B. Structures of methylenomycin A & B. J. Antibiot. 27:393-399.
119. **Harold, R. J., and D. A. Hopwood.** 1970. Ultraviolet-sensitive mutants of *Streptomyces coelicolor*. II. Genetics. Mutat. Res. 10:439-448.
120. **Harold, R. J., and D. A. Hopwood.** 1972. A rapid method for complementation testing of ultraviolet-sensitive (*uvs*) mutants of *Streptomyces coelicolor*. Mutat. Res. 16:27-34.

121. **Hastie, A. C.** 1970. Benlate-induced instability of *Aspergillus* diploids. Nature (London) 226:771.
122. **Higgens, C. E., R. L. Hamill, T. H. Sands, M. M. Hoehn, N. E. Davis, R. Nagarajan, and L. D. Boeck.** 1974. The occurrence of deacetoxycephalosporin C in fungi and streptomycetes. J. Antibiot. 27:298-300.
123. **Hikino, H., Y. Asada, S. Arihara, and T. Takemoto.** 1972. Fusidic acid, a steroidal antibiotic from *Isaria kogana*. Chem. Pharm. Bull. 20:1067-1069.
124. **Hirai, K., S. Nozoe, K. Tsuda, Y. Iitaka, K. Ishibashi, and M. Shirasaka.** 1967. The structure of siccanin. Tetrahedron Lett. 23:2177-2179.
125. **Hogg, R. W., and H. P. Broquist.** 1968. Homocitrate formation in *Neurospora crassa*. Relation to lysine biosynthesis. J. Biol. Chem. 243:1839-1845.
126. **Holloway, B. W.** 1975. Genetic organization in *Pseudomonas*, p. 133-161. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of pseudomonas. Wiley, London.
127. **Holt, G., and K. D. Macdonald.** 1968. Penicillin production and its mode of inheritance in *Aspergillus nidulans*. Antonie Van Leeuwenhoek J. Microbiol. Serol. 34:409-416.
128. **Holt, G., and K. D. Macdonald.** 1968. Isolation of strains with increased penicillin yield after hybridisation in *Aspergillus nidulans*. Nature (London) 219:636-637.
129. **Holt, G., G. F. St. L. Edwards, and K. D. Macdonald.** 1976. The genetics of mutants impaired in the biosynthesis of penicillin, p. 199-211. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
130. **Hopwood, D. A.** 1959. Linkage and the mechanism of recombination in *Streptomyces coelicolor*. Ann. N.Y. Acad. Sci. 81:887-898.
131. **Hopwood, D. A.** 1965. New data on the linkage map of *Streptomyces coelicolor*. Genet. Res. 6:248-262.
132. **Hopwood, D. A.** 1966. Lack of constant genome ends in *Streptomyces coelicolor*. Genetics 54:1177-1184.
133. **Hopwood, D. A.** 1967. Genetic analysis and genome structure in *Streptomyces coelicolor*. Bacteriol. Rev. 31:373-403.
134. **Hopwood, D. A.** 1969. Genome topology and mapping in *Streptomyces coelicolor*, p. 5-18. *In* G. Sermonti and M. Alačević (ed.), Genetics and breeding in streptomyces. Yugoslav Academy of Sciences and Arts, Zagreb.
135. **Hopwood, D. A.** 1972. Genetic analysis in microorganisms, p. 29-159. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 7B. Academic Press, London.
136. **Hopwood, D. A.** 1973. Developments in actinomycete genetics, p. 21-46. *In* Z. Vaněk, Z. Hošťálek, and J. Cudlín (ed.), Genetics of industrial microorganisms: actinomycetes and fungi. Academia, Prague.
137. **Hopwood, D. A.** 1973. Genetics of the actinomycetales, p. 131-153. *In* G. Sykes and F. A. Skinner (ed.), Actinomycetales: characteristics and practical importance. Academic Press, London.
138. **Hopwood, D. A.** 1977. Genetic recombination and strain improvement. Dev. Ind. Microbiol. 18:111-123.
139. **Hopwood, D. A., and K. F. Chater.** 1974. *Streptomyces coelicolor*, p. 237-255. *In* R. C. King (ed.), Handbook of genetics, vol. 1. Plenum Press, New York and London.
140. **Hopwood, D. A., K. F. Chater, J. E. Dowding, and A. Vivian.** 1973. Advances in *Streptomyces coelicolor* genetics. Bacteriol Rev. 37:371-405.
141. **Hopwood, D. A., and G. Sermonti.** 1962. The genetics of *Streptomyces coelicolor*. Adv. Genet. 11:273-342.
142. **Hopwood, D. A., and H. M. Wright.** 1972. Transformation in *Thermoactinomyces vulgaris*. J. Gen. Microbiol. 71:383-398.
143. **Hopwood, D. A., and H. M. Wright.** 1973. Transfer of a plasmid between streptomyces species. J. Gen. Microbiol. 77:187-195.
144. **Hopwood, D. A., and H. M. Wright.** 1973. A plasmid of *Streptomyces coelicolor* carrying a chromosomal locus and its inter-specific transfer. J. Gen. Microbiol. 79:331-342.
145. **Hopwood, D. A., and H. M. Wright.** 1976. Interactions of the plasmid SCP1 with the chromosome of *Streptomyces coelicolor* A3(2), p. 607-619. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
146. **Hopwood, D. A., and H. M. Wright.** 1976. Genetic studies on SCP1-prime strains of *Streptomyces coelicolor* A3(2). J. Gen. Microbiol. 95:107-120.
146a. **Hopwood, D. A., H. M. Wright, M. J. Bibb, and S. N. Cohen.** 1977. Genetic recombination through protoplast fusion in *Streptomyces*. Nature (London) 268:171-174.
147. **Hošťálek, Z., M. Blumauerovà, and Z. Vaněk.** 1974. Genetic problems of the biosynthesis of tetracycline antibiotics, p. 13-67. *In* T. K. Ghose, A. Fiechter, and N. Blakebrough (ed.), Advances in biochemical engineering 3. Springer-Verlag, Berlin, Heidelberg, and New York.
148. **Houghton, J. A.** 1970. A new class of slow-growing non-perithecial mutants of *Aspergillus nidulans*. Genet. Res. 16:285-292.
149. **Iwaki, M., K. Shimura, M. Kanda, E. Kaji, and Y. Saito.** 1972. Some mutants of *Bacillus brevis* deficient in gramicidin S formation. Biochem. Biophys. Res. Commun. 48:113-118.
150. **Jackson, M., E. L. Dulaney, I. Putter, H. M. Shafer, F. J. Wolf, and H. B. Woodruff.** 1962. Transethylation in anitbiotic biosynthesis II. Production of the 2'-ethoxy analogue of griseofulvin by biosynthesis. Biochim. Biophys. Acta 62:616-619.
151. **Jinks, J. L.** 1954. Somatic selection in fungi. Nature (London) 174:409-410.

152. **Jinks, J. L., C. E. Caten, G. Simchen, and J. H. Croft.** 1966. Heterokaryon incompatibility and variation in wild populations of *Aspergillus nidulans*. Heredity 21:227-239.
153. **Jinks, J. L., and V. Connolly.** 1975. Determination of the environmental sensitivity of selection lines by the selection environments. Heredity 34:401-406.
154. **Jones, A., and D. W. S. Westlake.** 1974. Regulation of chloramphenicol synthesis in *Streptomyces* sp. 3022a. Properties of arylamine synthetase, an enzyme involved in antibiotic biosynthesis. Can. J. Microbiol. 20:1599-1611.
155. **Käfer, E.** 1958. An eight chromosome map of *Aspergillus nidulans*. Adv. Genet. 9:105-145.
156. **Käfer, E.** 1965. Origins of translocations in *Aspergillus nidulans*. Genetics 52:217-232.
157. **Kähler, R., and D. Noack.** 1974. Action of acridine orange and ethidium bromide on growth and antibiotic activity of *Streptomyces hygroscopicus* JA 6599. Z. Allg. Mikrobiol. 14:529-533.
158. **Kambe, M., Y. Imae, and K. Kurahashi.** 1974. Biochemical studies on gramicidin S nonproducing mutants of *Bacillus brevis* ATCC 9999. J. Biochem. (Tokyo) 75:481-493.
159. **Kanzaki, T., and Y. Fujisawa.** 1975. Recent progress in cephalosporin fermentation. J. Takeda Res. Lab. 34:324-349.
160. **Kanzaki, T., T. Fukita, H. Shirafuji, Y. Fujisawa, and K. Kitano.** 1974. Occurrence of a 3-methylthiomethylcephem derivative in a culture broth of *Cephalosporium* mutant. J. Antibiot. 27:361-362.
161. **Kawamoto, I., S. Takasawa, R. Okashi, M. Kohakura, I. Takahashi, and T. Nara.** 1975. A new antibiotic victomycin (XK 49-1-B-2). I. Taxonomy and production of the producing organism. J. Antibiot. 28:358-365.
162. **Khua-ló, L.** 1962. Hybridisation in *Actinomyces erythreus*. (in Russian). Mikrobiologiya 31:61-65.
165. **Kirby, R., and D. A. Hopwood.** 1977. Genetic determination of methylenomycin synthesis by the SCP1 plasmid of *Streptomyces coelicolor* A3(2). J. Gen. Microbiol. 98:239-252.
166. **Kirby, R., L. F. Wright, and D. A. Hopwood.** 1975. Plasmid-determined antibiotic synthesis and resistance in *Streptomyces coelicolor*. Nature (London) 254:265-267.
167. **Kohsaka, M., and A. L. Demain.** 1976. Conversion of penicillin N to cephalosporin(s) by cell-free extracts of *Cephalosporium acremonium*. Biochem. Biophys. Res. Commun. 70:465-473.
168. **Kojima, M., and A. Satoh.** 1973. Microbial semi-synthesis of aminoglycosidic antibiotics by mutants of *S. ribosificus* and *S. kanamyceticus*. J. Antibiot. 26:784-786.
169. **Kominek, L. A., and O. K. Sebek.** 1974. Biosynthesis of novobiocin and related antibiotics. Dev. Ind. Microbiol. 15:60-69.
170. **Kozak, W., M. Rajchert-Trzpil, and W. T. Dobrzanski.** 1974. The effect of proflavin, ethidium bromide and an elevated temperature on the appearance of nisin-negative clones in nisin-producing strains of *Streptococcus lactis*. J. Gen. Microbiol. 83:295-302.
171. **Kutzner, H. J., and S. A. Waksman.** 1959. *Streptomyces coelicolor* Müller and *Streptomyces violaceoruber* Waksman and Curtis, two distinctly different organisms. J. Bacteriol. 78:528-538.
172. **Laland, S. G., and T. Zimmer.** 1973. The protein thiotemplate mechanism of synthesis for the peptide antibiotics produced by *Bacillus brevis*. Essays Biochem. 9:31-57.
173. **Lancini, G. C., and R. J. White.** 1976. Rifamycin biosynthesis, p. 139-153. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
173a. **Lemke, P. A., and C. H. Nash.** 1972. Mutations that affect antibiotic synthesis by *Cephalosporium acremonium*. Can. J. Microbiol. 18:255-259.
174. **Lepesant-Kejzlarovà, J., J.-A. Lepesant, J. Walle, A. Billault, and R. Dedonder.** 1975. Revision of the linkage map of *Bacillus subtilis* 168: indications for circularity of the chromosome. J. Bacteriol. 121:823-834.
175. **Lewin, B.** 1974. Gene expression. 1. Bacterial genomes. John Wiley & Sons, London and New York.
176. **Lewin, B.** 1974. Gene expression. 2. Eucaryotic chromosomes. John Wiley & Sons, London and New York.
177. **Lhoas, P.** 1961. Mitotic haploidization by treatment of *Aspergillus niger* diploids with parafluorophenylalanine. Nature (London) 190:744.
178. **Lhoas, P.** 1967. Genetic analysis by means of the parasexual cycle in *Aspergillus niger*. Genet. Res. 10:45-61.
179. **Liersch, M., J. Nüesch, and H. J. Treichler.** 1976. Final steps in the biosynthesis of cephalosporin C, p. 179-195. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
180. **Lipmann, F.** 1973. Nonribosomal polypeptide synthesis on polyenzyme templates. Acc. Chem. Res. 6:361-367.
181. **Loder, P. B., and E. P. Abraham.** 1971. Isolation and nature of intracellular peptides from a cephalosporin C-producing *Cephalosporium* sp. Biochem. J. 123:471-476.
182. **Lomovskaya, N. D., T. A. Voeykova, and N. M. Mkrtumian.** 1977. Construction and properties of hybrids obtained in interspecific crosses between *Streptomyces coelicolor* A3(2) and *Streptomyces griseus* Kr.15. J. Gen. Microbiol. 98:187-198.
183. **MacDonald, J. C.** 1967. Pyocyanine, p. 52-65. *In* D. Gottlieb and P. D. Shaw (ed.). Antibiotics: biosynthesis, vol. 2. Springer-Verlag, Berlin, Heidelberg, and New York.

184. **Macdonald, K. D.** 1966. Differences in diploids synthesised between the same parental strains of *Penicillium chrysogenum*. Antonie van Leeuwenhoek J. Microbiol. Serol. 32:431-441.
185. **Macdonald, K. D.** 1968. The persistence of parental genome segregation in *Penicillium chrysogenum* after nitrogen mustard treatment. Mutat. Res. 5:302-305.
186. **Macdonald, K. D.** 1971. Segregants from a heterozygous diploid of *Penicillium chrysogenum* following different physical and chemical treatments. J. Gen. Microbiol. 67:247-250.
187. **Macdonald, K. D., and G. Holt.** 1976. Genetics of biosynthesis and overproduction of penicillin. Sci. Prog. (London) 63:547-573.
188. **Macdonald, K. D., G. Holt, and P. Ditchburn.** 1972. The genetics of penicillin production, p. 251-257. Proceedings of the Fourth International Fermentation Symposium: Fermentation Technology Today. Society for Fermentation Technology, Osaka.
189. **Macdonald, K. D., J. M. Hutchinson, and W. A. Gillett.** 1963. Isolation of auxotrophs of *Penicillium chrysogenum* and their penicillin yields. J. Gen. Microbiol. 33:365-374.
190. **Macdonald, K. D., J. M. Hutchinson, and W. A. Gillett.** 1963. Heterokaryon studies and the genetic control of penicillin and chrysogenin production in *Penicillium chrysogenum*. J. Gen. Microbiol. 33:375-383.
191. **Macdonald, K. D., J. M. Hutchinson, and W. A. Gillett.** 1963. Formation and segregation of heterozygous diploids between a wild-type strain and derivatives of high penicillin yield in *Penicillium chrysogenum*. J. Gen. Microbiol. 33:385-394.
192. **Macdonald, K. D., J. M. Hutchinson, and W. A. Gillett.** 1964. Properties of heterozygous diploids between strains of *Penicillium chrysogenum* selected for high penicillin yield. Antonie van Leeuwenhoek J. Microbiol. Serol. 30:209-224.
193. **Macdonald, K. D., J. M. Hutchinson, and W. A. Gillett.** 1965. Heterozygous diploids of *Penicillium chrysogenum* and their segregation patterns. Genetica 36:378-397.
194. **Majer, J., J. R. Martin, R. S. Egan, and J. W. Corcoran.** 1977. Antibiotic glycosides. VIII. Erythromycin D, a new macrolide antibiotic. J. Am. Chem. Soc. 99:1620-1622.
195. **Maragoudakis, M. E., H. Holmes, and M. Strassman.** 1967. Control of lysine biosynthesis in yeast by a feedback mechanism. J. Bacteriol. 93:1677-1680.
196. **Martin, J. R., R. S. Egan, A. W. Goldstein, and P. Collum.** 1975. Extension of the erythromycin biosynthetic pathway. Isolation and structure of erythromycin E. Tetrahedron 31:1985-1989.
197. **Martin, J. R., and T. J. Perun.** 1968. Studies on the biosynthesis of the erythromycins. III. Isolation and structure of 5-deoxy-5-oxoerythronolide B, a shunt metabolite of erythromycin biosynthesis. Biochemistry 7:1728-1733.
198. **Martin, J. R., T. J. Perun, and R. L. Girolami.** 1966. Studies on the biosynthesis of the erythromycins. I. Isolation and structure of an intermediate glycoside, 3-α-L-mycarosylerythronolide B. Biochemistry 5:2852-2856.
199. **Martin, J. R., and W. Rosenbrook.** 1967. Studies on the biosynthesis of the erythromycins. II. Isolation and structure of a biosynthetic intermediate, 6-deoxyerythronolide B. Biochemistry 6:435-440.
200. **Masurekar, P. S., and A. L. Demain.** 1974. Insensitivity of homocitrate synthase in extracts of *Penicillium chrysogenum* to feedback inhibition by lysine. Appl. Microbiol. 28:265-270.
201. **Masurekar, P. S., and A. L. Demain.** 1974. Impaired penicillin production in lysine regulatory mutants of *Penicillium chrysogenum*. Antimicrob. Agents Chemother. 6:366-368.
202. **Mather, K.** 1942. The balance of polygenic combinations. J. Genet. 43:309-336.
203. **Mather, K.** 1949. Biometrical genetics, 1st ed. Methuen, London.
204. **Mather, K., and J. L. Jinks.** 1971. Biometrical genetics, 2nd ed. Chapman and Hall, London.
205. **Matselyukh, B. P.** 1976. Structure and function of the *Actinomyces olivaceus* genome, p. 553-563. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
206. **McCormick, J. R. D.** 1969. Point-blocked mutants and the biogenesis of tetracyclines, p. 163-176. *In* G. Sermonti and M. Alačević (ed.), Genetics and breeding of streptomyces. Yugoslav Academy of Sciences and Arts, Zagreb.
207. **McCully, K. S., and E. Forbes.** 1965. The use of *p*-fluorophenylalanine with 'master strains' of *Aspergillus nidulans*. Genet. Res. 6:352-359.
208. **Merrick, M. J.** 1975. Hybridisation and selection for increased penicillin titre in wild-type isolates of *Aspergillus nidulans*. J. Gen. Microbiol. 91:278-286.
209. **Merrick, M. J.** 1975. The inheritance of penicillin titre in crosses between lines of *Aspergillus nidulans* selected for increased productivity. J. Gen. Microbiol. 91:287-294.
210. **Merrick, M. J.** 1976. Hybridization and selection for penicillin production in *Aspergillus nidulans*—a biometrical approach to strain improvement, p. 229-242. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
211. **Merrick, M. J.** 1976. A morphological and genetic mapping study of bald colony mutants of *Streptomyces coelicolor*. J. Gen. Microbiol. 96:299-315.

212. **Merrick, M. J., and C. E. Caten.** 1975. The inheritance of penicillin titre in wild-type isolates of *Aspergillus nidulans*. J. Gen. Microbiol. 86:283–293.
213. **Meynell, G. G.** 1972. Bacterial plasmids. Macmillan, London.
214. **Mindlin, S. Z.** 1969. Genetic recombination in the actinomycete breeding, p. 147–159. *In* G. Sermonti and M. Alačević (ed.), Genetics and breeding of streptomyces. Yugoslav Academy of Sciences and Arts, Zagreb.
215. **Miniken, D. E., and M. Goodfellow.** 1976. Lipid composition in the classification and identification of nocardiae and related taxa, p. 160–219. *In* M. Goodfellow, G. H. Brownell, and J. A. Serrano (ed.), The biology of the nocardiae. Academic Press, London, New York, and San Francisco.
216. **Murphy, G., and F. Lynen.** 1975. Patulin biosynthesis: the metabolism of *m*-hydroxybenzyl alcohol and *m*-hydroxybenzaldehyde by particulate preparations from *Penicillium patulum*. Eur. J. Biochem. 58:467–475.
217. **Musílek, V., J. Čerrá, V. Šašek, M. Semerdžieva, and M. Vondraček.** 1969. Antifungal antibiotic of the basidiomycete *Oudemanciella mucida*. I. Isolation and cultivation of a producing strain. Folia Microbiol. (Prague) 14:377–387.
218. **Nagaoka, K., and A. L. Demain.** 1975. Mutational biosynthesis of a new antibiotic, streptomutin A, by an idiotroph of *Streptomyces griseus*. J. Antibiot. 28:627–635.
219. **Nagarajan, R., L. D. Boeck, M. Gorman, R. L. Hamill, C. E. Higgens, M. N. Hoehn, W. M. Stark, and J. G. Whitney.** 1971. β-lactam antibiotics from streptomyces. J. Am. Chem. Soc. 93:2308–2310.
220. **Nash, C. H.,N. de la Higuera, N. Neuss, and P. A. Lemke.** 1974. Application of biochemical genetics to the biosynthesis of β-lactam antibiotics. Dev. Ind. Microbiol. 15:114–132.
221. **Nash, C. H., and F. M. Huber.** 1971. Antibiotic synthesis and morphological differentiation of *Cephalosporium acremonium*. Appl. Microbiol. 22:6–10.
222. **North, J.** 1976. The effect of griseofulvin on diploid strains of *Coprinus lagopus*. J. Gen. Microbiol. 98:529–534.
223. **Nüesch, J., H. J. Treichler, and M. Liersch.** 1973. The biosynthesis of cephalosporin C, p. 309–334. *In* Z. Vaněk, Z. Hoštálek, and J. Cudlín (ed.), Genetics of industrial microorganisms: actinomycetes and fungi. Academia, Prague.
224. **Okanishi, M., K. Hamana, and H. Umezawa.** 1968. Factors affecting infection of protoplasts with deoxyribonucleic acid of actinophage PK-66. J. Virol. 2:686–691.
225. **Okanishi, M., T. Ohta, and H. Umezawa.** 1970. Possible control of formation of aerial mycelium and antibiotic production in *Streptomyces* by episomic factors. J. Antibiot. 23:45–47.
226. **Okanishi, M., K. Suzuki, and H. Umezawa.** 1974. Formation and reversion of streptomycete protoplasts: cultural condition and morphological study. J. Gen. Microbiol. 80:389–400.
227. **Okanishi, M., R. Utahara, and Y. Okami.** 1966. Infection of the protoplasts of *Streptomyces kanamyceticus* with deoxyribonucleic acid preparation from actinophage PK-66. J. Bacteriol. 92:1850–1852.
228. **Ōmura, S.** 1976. The antibiotic cerulenin, a novel tool for biochemistry as an inhibitor of fatty acid synthesis. Bacteriol. Rev. 40:681–697.
229. **O'Sullivan, C. Y., and S. J. Pirt.** 1973. Penicillin production by lysine auxotrophs of *Penicillium chrysogenum*. J. Gen. Microbiol. 76:65–75.
230. **Papa, K. E.** 1971. Growth rate in *Neurospora crassa*: linkage of polygenes. Genetica 42:181–186.
231. **Perlman, D.** 1974. Prospects for the fermentation industries, 1974–1983. Chem. Technol. 4:210–216.
232. **Peterson, E. A., D. C. Gillespie, and F. D. Cook.** 1966. A wide-spectrum antibiotic produced by a species of sorangium. Can. J. Microbiol. 12:221–230.
233. **Pirali, G., S. Somma, G. C. Lancini, and F. Sala.** 1974. Inhibition of peptide chain initiation in *Escherichia coli* by thermorubin. Biochim. Biophys. Acta 366:310–318.
234. **Pirt, S. J.** 1969. Microbial growth and product formation. Symp. Soc. Gen. Microbiol. 19:199–221.
235. **Polsinelli, M., and M. Beretta.** 1966. Genetic recombination in crosses between *Streptomyces aureofaciens* and *Streptomyces rimosus*. J. Bacteriol. 91:63–68.
236. **Pontecorvo, G.** 1976. Presidential address, p. 1–4. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
237. **Pontecorvo, G., and E. Käfer.** 1958. Genetic analysis based on mitotic recombination. Adv. Genet. 9:71–104.
238. **Pontecorvo, G., J. A. Roper, L. M. Hemmons, K. D. Macdonald, and A. Bufton.** 1953. The genetics of *Aspergillus nidulans*. Adv. Genet. 5:141–253.
239. **Pontecorvo, G., and G. Sermonti.** 1954. Parasexual recombination in *Penicillium chrysogenum*. J. Gen. Microbiol. 11:94–104.
240. **Puglia, A. M., I. Spada-Sermonti, S. Basile, F. Misuraca, and G. Sermonti.** 1973. Infectious transfer of a fertility factor in *Streptomyces coelicolor*. Genet. Res. 21:107–113.
241. **Queener, S. W., J. J. Capone, A. B. Radue, and R. Nagarajan.** 1974. Synthesis of deacetoxycephalosporin C by a mutant of *Cephalosporium acremonium*. Antimicrob. Agents Chemother. 6:334–337.
242. **Queener, S. W., J. McDermott, and A. B. Radue.** 1975. Glutamate dehydrogenase specific activity and cephalosporin C synthesis

in the M8650 series of *Cephalosporium acremonium* mutants. Antimicrob. Agents Chemother. 7:646-651.

243. **Redshaw, P. A., P. A. McCann, L. Sankaran, and B. M. Pogell.** 1976. Control of differentiation in streptomycetes: involvement of extrachromosomal deoxyribonucleic acid and glucose repression in aerial mycelia development. J. Bacteriol. 125:698-705.
244. **Reeves, P.** 1972. The bacteriocins. Chapman and Hall Ltd., London.
245. **Rickenberg, H. V.** 1974. Cyclic AMP in prokaryotes. Annu. Rev. Microbiol. 28:353-369.
246. **Rinehart, K. L., and R. M. Stroshane.** 1976. Biosynthesis of aminocyclitol antibiotics. J. Antibiot. 29:319-353.
247. **Roland, I., Ø. Frøyshov, and S. G. Laland.** 1975. On the presence of pantothenic acid in the three complementary enzymes of bacitracin synthetase. FEBS Lett. 60:305-308.
248. **Roper, J. A.** 1952. Production of heterozygous diploids in filamentous fungi. Experientia 8:14-15.
249. **Russi, S., A. Carere, B. Fratello, and V. Khoudokormoff.** 1966. Caratterizzazione biochimica di alcuni mutanti di *Streptomyces coelicolor* richiedenti istidina. Ann. Ist. Super. Sanita 2:506-522.
250. **Sadoff, H. L.** 1972. Sporulation antibiotics of *Bacillus* species, p. 157-166. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.
251. **Saito, H., and Y. Ikeda.** 1959. Cytogenetic studies on *Streptomyces griseoflavus*. Ann. N.Y. Acad. Sci. 81:862-878.
252. **Sankaran, L., and B. M. Pogell.** 1975. Biosynthesis of puromycin in *Streptomyces alboniger*: regulation and properties of O-demethylpuromycin O-methyltransferase. Antimicrob. Agent Chemother. 8:721-732.
253. **Schaeffer, P.** 1969. Sporulation and the production of antibiotics, exoenzymes and exotoxins. Bacteriol. Rev. 33:48-71.
254. **Schier, W. T., S. Ogawa, M. Hichens, and K. L. Rinehart.** 1973. Chemistry and biochemistry of the neomycins. XVII. Bioconversion of aminocyclitols to aminocyclitol antibiotics. J. Antibiot. 26:551-561.
255. **Schier, W. T., K. L. Rinehart, and D. Gottlieb.** 1969. Preparation of four new antibiotics from a mutant of *Streptomyces fradiae*. Proc. Natl. Acad. Sci. U.S.A. 63:198-204.
256. **Schier, W. T., P. C. Schaefer, D. Gottlieb, and K. L. Rinehart.** 1974. Use of mutants in the study of aminocyclitol antibiotic biosynthesis and the preparation of the hybrimycin C complex. Biochemistry 13:5073-5078.
257. **Schrempf, H., H. Bujard, D. A. Hopwood, and W. Goebel.** 1975. Isolation of covalently closed circular deoxyribonucleic acid from *Streptomyces coelicolor* A3(2). J. Bacteriol. 121:416-421.
258. **Schupp, T.** 1976. Genetic analysis in *Nocardia mediterranei*, p. 531-533. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
259. **Schupp, T., R. Hütter, and D. A. Hopwood.** 1975. Genetic recombination in *Nocardia mediterranei*. J. Bacteriol. 121:128-136.
260. **Sermonti, G.** 1956. Complementary genes which affect penicillin yields. J. Gen. Microbiol. 15:599-608.
261. **Sermonti, G.** 1957. Produzione di penicillina da diploidi eterozigoti di *Penicillium chrysogenum*. Conv. Genetica 1956 Ricerca Sci. 27:93-100.
262. **Sermonti, G.** 1959. Genetics of penicillin production. Ann. N.Y. Acad. Sci. 81:950-973.
263. **Sermonti, G.** 1961. The parasexual cycle in *Penicillium chrysogenum* and its application to the production of penicillin. Sci. Rep. Ist. Super. Sanita. 1:441.
264. **Sermonti, G.** 1969. Genetics of antibiotic-producing microorganisms. Wiley-Interscience, London.
265. **Sermonti, G., M. Bandiera, and I. Spada-Sermonti.** 1966. New approach to the genetics of *Streptomyces coelicolor*. J. Bacteriol. 91:384-392.
266. **Sermonti, G., A. Mancinelli, and I. Spada-Sermonti.** 1960. Heterogeneous clones ("heteroclones") in *Streptomyces coelicolor* A3(2). Genetics 45:669-672.
267. **Sermonti, G., and A. M. Puglia.** 1976. Progressive fertilization in *Streptomyces coelicolor*, p. 565-572. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.
268. **Sermonti, G., A. M. Puglia, and G. Ficarra.** 1971. The time course of recombinant production in *Streptomyces coelicolor*. Genet. Res. 18:133-144.
269. **Shimura, K., M. Iwaki, M. Kanda, K. Hori, E. Kaji, S. Hasegawa, and Y. Saito.** 1974. On the enzyme system obtained from some mutants of *Bacillus brevis* deficient in gramicidin S formation. Biochim. Biophys. Acta 338:577-587.
270. **Silvestri, L. G.** 1970. The evolution of the thermophilic *Actinomycetales*: an apparent evolutionary paradox, p. 239-243. *In* H. Prauser (ed.), The Actinomycetales, the Jena International Symposium on Taxonomy, 1968. Gustav Fischer Verlag, Jena.
271. **Singh, J.** 1967. Patulin, p. 621-630. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics, vol. 1. Springer-Verlag, Berlin, Heidelberg, and New York.
272. **Smith, B., S. C. Warren, G. G. F. Newton, and E. P. Abraham.** 1967. Biosynthesis of penicillin N and cephalosporin C. Biochem. J. 103:877-890.
273. **Somerson, N. L., A. L. Demain, and T. D. Nunheimer.** 1961. Reversal of lysine inhibition of penicillin production by α-aminoadipic acid. Arch. Biochem. Biophys. 93:238.

274. **Stanisich, V. A., and M. H. Richmond.** 1975. Gene transfer in the genus *Pseudomonas*, p. 163-190. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of pseudomonas. John Wiley & Sons, London and New York.

275. **Stapley, E. O., M. Jackson, S. Hernandez, S. B. Zimmerman, S. A. Currie, S. Mochales, J. M. Mata, H. B. Woodruff, and D. Hendlin.** 1972. Cephamycins, a new family of β-lactam antibiotics. I. Production by actinomycetes, including *Streptomyces lactamdurans* sp. n. Antimicrob. Agents Chemother. 2:122-131.

276. **Stauffer, J. F., and M. P. Backus.** 1954. Spontaneous and induced variation in selected stocks of the *Penicillium chrysogenum* series. Ann. N.Y. Acad. Sci. 60:35-50.

277. **Tagg, J. R., A. S. Dajoni, and L. W. Wannamaker.** 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev. 40:722-756.

278. **Takasawa, S., I. Kawamoto, I. Takahashi, M. Kohakura, R. Okachi, S. Sato, M. Yamomoto, T. Sato, and T. Nara.** 1975. Platomycins A and B. I. Taxonomy of the producing strain and production, isolation and biological properties of platomycins. J. Antibiot. 28:656-661.

279. **Tanaka, N.** 1967. Variotin, p. 216-221. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics: biosynthesis, vol. 2. Springer-Verlag, Berlin, Heidelberg, and New York.

280. **Tanaka, H., Y. Koyama, T. Nagai, H. Marumo, and S. Ōmura.** 1975. Nanaomycins, new antibiotics produced by a strain of *Streptomyces*. II. Structure and biosynthesis. J. Antibiot. 28:868-875.

281. **Tardrew, P. L., and M. J. Johnson.** 1958. Sulfate utilization by penicillin-producing mutants of *Penicillium chrysogenum*. J. Bacteriol. 76:400-405.

282. **Taylor, H. D., and H. Schmitz.** 1976. Antibiotics derived from a mutant of *Bacillus circulans*. J. Antibiot. 29:532-535.

283. **Testa, R. T., G. H. Wagman, P. J. L. Daniels, and M. J. Weinstein.** 1974. Mutamicins: biosynthetically created new sisomicin analogues. J. Antibiot. 27:917-921.

284. **Tokunaga, T., Y. Mizuguchi, and K. Suga.** 1973. Genetic recombination in mycobacteria. J. Bacteriol. 113:1104-1111.

285. **Traxler, P. H., J. Treichler, and J. Nüesch.** 1975. Synthesis of *N*-acetyldeacetoxycephalosporin C by a mutant of *Cephalosporium acremonium*. J. Antibiot. 28:605-606.

286. **Turner, W. B.** 1971. Fungal metabolites. Academic Press, London and New York.

287. **Umezawa, H.** 1967. Index of antibiotics from actinomycetes. Pennsylvania State University Press, University Park.

288. **Upshall, A., and E. Käfer.** 1974. Detection and identification of translocations by increased specific nondisjunction in *Aspergillus nidulans*. Genetics 76:19-31.

289. **Vaks, B., A. Zuckerberg, and E. Rosenberg.** 1974. Purification and partial characterisation of an antibiotic produced by *Myxococcus xanthus*. Can. J. Microbiol. 20:155-161.

290. **Vanderhaeghe, H., P. Van Dijck, and P. de Somer.** 1965. Identity of ramycin with fusidic acid. Nature (London) 205:710-711.

291. **Vaněk, Z., and J. Majer.** 1967. Macrolide antibiotics, p. 154-188. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics: biosynthesis, vol. 2. Springer-Verlag, New York.

292. **Vining, L. C., V. S. Malik, and D. W. S. Westlake.** 1968. Biosynthesis of chloramphenicol. Lloydia 31:355-363.

293. **Vivian, A.** 1971. Genetic control of fertility in *Streptomyces coelicolor* A3(2): plasmid involvement in the interconversion of UF and IF strains. J. Gen. Microbiol. 69:353-364.

294. **Vladimirov, A. V.** 1966. Genetic recombination in *Actinomyces antibioticus* producing oleandomycin (in Russian). Antibiotiki (Moscow) 2:117.

295. **Walker, J. B.** 1971. Enzymatic reactions involved in streptomycin biosynthesis and metabolism. Lloydia 34:363-371.

296. **Walker, J. B.** 1967. Enzymatic studies on the biosynthesis of streptomycin and related antibiotics. Dev. Ind. Microbiol. 8:109-113.

297. **Watson, J. D.** 1976. Molecular biology of the gene, 3rd ed. W. A. Benjamin, Inc., Menlo Park, Calif.

298. **Weinberg, E. D.** 1974. Secondary metabolism: control by temperature and inorganic phosphate. Dev. Ind. Microbiol. 15:70-81.

299. **Wheelis, M. L.** 1975. The genetics of dissimilatory pathways in *Pseudomonas*. Annu. Rev. Microbiol. 29:505-523.

300. **White, R. J., E. Martinelli, G. G. Gallo, G. Lancini, and P. Beynon.** 1973. Rifamycin biosynthesis studied with ^{13}C-enriched precursors and carbon magnetic resonance. Nature (London) 243:273-277.

301. **White, R. J., E. Martinelli, and G. Lancini.** 1974. Ansamycin biogenesis: studies on a novel rifamycin isolated from a mutant strain of *Nocardia mediterranei*. Proc. Natl. Acad. Sci. U.S.A. 71:3260-3264.

302. **Wiame, J. M., and E. L. Dubois.** 1976. The regulation of enzyme synthesis in arginine metabolism of *Saccharomyces cerevisiae*, p. 391-406. *In* K. D. Macdonald (ed.), Second International Symposium on the Genetics of Industrial Microorganisms. Academic Press, London, New York, and San Francisco.

303. **Wildermuth, H.** 1970. Surface structure of streptomycete spores as revealed by negative staining and freeze-etching. J. Bacteriol. 101:318-322.

304. **Williams, R. P., and W. R. Hearn.** 1967. Prodigiosin, p. 410-432. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics: biosynthesis, vol. 2. Springer-Verlag, Berlin, Heidelberg, and New York.

305. **Williams, S. T., and M. R. Khan.** Antibiotics—a soil microbiologist's view. Postepy Hig. Med. Dosw. 28:395-408.

306. **Williams, S. T., G. P. Sharples, and R. M. Bradshaw.** 1973. The fine structure of the actinomycetales, p. 113-130. *In* G. Sykes and F. A. Skinner (ed.), Actinomycetales: characteristics and practical importance. Academic Press, London, and New York.

307. **Wright, L. F., and D. A. Hopwood.** 1976. Identification of the antibiotic determined by the SCP1 plasmid of *S. coelicolor* A3(2). J. Gen. Microbiol. **95**:96-106.

308. **Wright, L. F., and D. A. Hopwood.** 1976. Actinorhodin is a chromosomally determined antibiotic in *Streptomyces coelicolor* A3(2). J. Gen. Microbiol. **96**:289-297.

309. **Young, F. E., and G. A. Wilson.** 1972. Genetics of *Bacillus subtilis* and other gram-positive sporulating bacilli, p. 77-106. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.

310. **Young, F. E., and G. A. Wilson.** 1974. *Bacillus subtilis*, p. 69-114. *In* R. C. King (ed.), Handbook of genetics, vol. 1. Plenum Press, New York and London.

311. **Zhukova, R. A., L. N. Lanskaya, and T. G. Gavryuchenkova.** 1973. Lethal and selective effect of polygenic antibiotics levonin and mycoheptin on their producing organisms (in Russian). Genetika **9**:69-77.